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(54) Title: INTEGRIN ANTAGONISTS

(57) Abstract: The present invention provides methods and compositions for inhibiting the biological activity of integrins, for inhibiting endothelial cell migration, and for inhibiting angiogenesis. In particular, the invention provides compositions comprising ADAM disintegrin domains and methods for using said compositions. In preferred embodiments the methods and compositions of the invention are used to inhibit angiogenesis and to treat diseases or conditions mediated by angiogenesis.

TITLE
INTEGRIN ANTAGONISTS

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 This application claims the benefit of pending U.S. provisional application Serial No. 60/184,865, filed 25 February 2000, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

- 10 This invention relates to methods and compositions that are useful for antagonizing the interaction between integrins and their ligands. In particular, the invention relates to the use of ADAM disintegrin domains for antagonizing the interaction between integrins and their ligands.

BACKGROUND OF THE INVENTION

A. Integrins and Disintegrins

- 15 Integrins are a family of cell surface proteins that mediate adhesion between cells (cell-cell adhesion) and between cells and extracellular matrix proteins (cell-ECM adhesion). Integrins are heterodimeric structures composed of noncovalently bound α and β subunits. In humans, at least fifteen different α subunits and eight different β subunits combine to form integrins with diverse biological activities and ligand specificities. Integrins play important roles in biological processes
20 including embryonic development, platelet aggregation, immune reactions, tissue repair and remodeling, bone resorption, and tumor invasion and metastasis. Integrins are, therefore, important targets for therapeutic intervention in human disease.

- The disintegrins are a family of low molecular weight, soluble, cysteine-rich peptides which have been isolated from snake venom (reviewed in Niewiarowski et al., Seminars in Hematology
25 31(4):289, 1994). The snake venom disintegrins typically contain an RGD (Arg-Gly-Asp, SEQ ID NO:19) motif. The RGD motif is recognized by many integrins, and is present in several integrin ligands including fibronectin, vitronectin, and von Willebrand factor. Disintegrins disrupt normal adhesion processes by inhibiting the binding of cell surface integrins to their ligands.

- Disintegrin-like domains have been identified in cellular proteins from both invertebrates and
30 vertebrates (see, e.g., Westcamp and Blobel, Proc. Natl. Acad. Sci. USA 91:2748, 1994; Wolfsberg et al., Dev. Biol. 169:378, 1995; Alfandari et al., Dev. Biol. 182:314, 1997), including the ADAM family of transmembrane proteins.

B. ADAMs

- 35 The ADAMs, which have also been called MDCs, are a family of type I transmembrane cysteine-rich glycoproteins (Weskamp et al., Proc. Natl. Acad. Sci. USA, 91:2748, 1994; Wolfsberg et al., Dev. Biol. 169:378, 1995). The multidomain structure of the ADAMs typically includes an amino-terminal metalloprotease domain, a disintegrin domain, a cysteine-rich region (the region between the

disintegrin domain and the transmembrane domain), a transmembrane region, and a cytoplasmic domain. At least 30 ADAM family members have been identified, in a variety of animal species. The structure of the ADAMs suggests that they may be involved in a variety of biological processes, including cell adhesion, cell fusion, signal transduction, and proteolysis. Members of the ADAM family have, in fact, been shown to play roles in sperm-egg binding and fusion, myotube formation, neurogenesis, and proteolysis.

ADAM-15, also called MDC-15 or metargidin, is the only ADAM identified to date which contains an RGD motif within its disintegrin domain. Zhang et al. (J. Biol. Chem. 273(13):7345, 1998) have reported that the isolated disintegrin domain of ADAM-15, expressed in *E. coli* as a glutathione S-transferase fusion protein, specifically interacts with $\alpha_v\beta_3$ integrin and that the interaction is mediated by the RGD tripeptide sequence. The recombinant fusion protein did not interact with other integrins tested, including $\alpha_{IIb}\beta_3$ and $\alpha_5\beta_1$. Nath et al. (J. Cell Science 112:579, 1999) have reported that the entire ADAM-15 extracellular domain, expressed as an Fc fusion protein in COS cells, interacts with $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins on hematopoietic cells and that the interaction is mediated by the RGD tripeptide sequence. Zhang et al. and Nath et al. commented that the RGD-dependent interaction between ADAM-15 and $\alpha_v\beta_3$ integrin suggests a role in processes such as malignancy and angiogenesis.

C. Angiogenesis

Angiogenesis, the generation of new blood vessels, is a spatially and temporally regulated process in which endothelial and smooth muscle cells proliferate, migrate, and assemble into tubes, in response to endogenous positive and negative regulatory molecules. Angiogenesis plays important roles in both normal and pathological physiology.

Under normal physiological conditions, angiogenesis is involved in fetal and embryonic development, wound healing, organ regeneration, and female reproductive remodeling processes including formation of the endometrium, corpus luteum, and placenta. Angiogenesis is stringently regulated under normal conditions, especially in adult animals, and perturbation of the regulatory controls can lead to pathological angiogenesis.

Pathological angiogenesis has been implicated in the manifestation and/or progression of inflammatory diseases, certain eye disorders, and cancer. In particular, several lines of evidence support the concept that angiogenesis is essential for the growth and persistence of solid tumors and their metastases (see, e.g., Folkman, N. Engl. J. Med. 285:1182, 1971; Folkman et al., Nature 339:58, 1989; Kim et al., Nature 362:841, 1993; Hori et al., Cancer Res., 51:6180, 1991; Zetter, Annu. Rev. Med. 49:407, 1998). The formation of new blood vessels provides a growing tumor with oxygen, nutrients, waste removal, and a conduit by which invasive cells can enter the circulatory system and establish distant metastases. Various classes of angiogenesis inhibitors are presently being developed and tested for the prevention (e.g., treatment of premalignant conditions), intervention (e.g., treatment of small tumors), and regression (e.g., treatment of large tumors) of cancers (see, e.g., Bergers et al.,

Science 284:808, 1999) and other forms of pathological angiogenesis. Because many steps in the angiogenic process, including endothelial cell migration, proliferation, and morphogenesis require vascular cell adhesion, certain integrin antagonists have been tested as anti-angiogenic agents.

Several integrins are expressed on the surface of cultured endothelial and smooth muscle cells, including $\alpha_v\beta_3$ integrin. The $\alpha_v\beta_3$ integrin is an endothelial cell receptor for von Willebrand factor, fibrin, fibrinogen, and fibronectin, and a marker of angiogenic vascular tissue. Brooks et al. have reported that monoclonal antibodies to $\alpha_v\beta_3$ integrin, as well as cyclic peptide inhibitors, disrupt angiogenesis and that $\alpha_v\beta_3$ antibodies promote tumor regression (Science 264:569, 1994; Cell 79:1157, 1994). These results suggest that $\alpha_v\beta_3$ integrin is a useful therapeutic target for diseases characterized by pathological angiogenesis.

There is great need for additional compositions and methods of antagonizing the interaction between integrins and their ligands. In particular, there is great need for additional compositions and methods of inhibiting angiogenesis for the prevention, abrogation, and mitigation of disease processes that are dependent upon pathological angiogenesis.

SUMMARY OF THE INVENTION

The present invention is based upon the discovery that ADAM disintegrin domains are useful for inhibiting the biological activity of integrins and for inhibiting endothelial cell migration and angiogenesis, including the unexpected discovery that these inhibitory activities reside in ADAM disintegrin domains that lack an RGD motif.

The invention is directed to methods of antagonizing the binding of an integrin to its ligands, and thereby inhibiting the biological activity of the integrin, comprising contacting the integrin with an effective amount of an ADAM disintegrin domain polypeptide. The invention is further directed to methods of inhibiting endothelial cell migration and methods of inhibiting angiogenesis comprising administering an effective amount of an ADAM disintegrin domain polypeptide. In some embodiments the ADAM disintegrin domain polypeptide is in the form of a multimer, preferably a leucine zipper multimer or Fc polypeptide. In some embodiments the ADAM disintegrin domain is from a human ADAM, and preferably from ADAM-8, ADAM-9, ADAM-10, ADAM-15, ADAM-17, ADAM-20, ADAM-21, ADAM-22, ADAM-23, or ADAM-29. The ADAM disintegrin domain is preferably produced in a recombinant cell, and is preferably present in a composition comprising a pharmaceutically acceptable carrier.

In some preferred embodiments the ADAM disintegrin domain polypeptide comprises an amino acid sequence selected from the group consisting of: amino acids 23-264 of SEQ ID NO:2, amino acids 23-303 of SEQ ID NO:4, amino acids 23-235 of SEQ ID NO:6, amino acids 23-292 of SEQ ID NO:8, amino acids 23-216 of SEQ ID NO:10, amino acids 23-305 of SEQ ID NO:12, amino acids 23-293 of SEQ ID NO:14, amino acids 23-312 of SEQ ID NO:16, amino acids 23-310 of SEQ ID NO:18, and amino acids 23-298 of SEQ ID NO:22. In some more preferred embodiments the ADAM disintegrin domain polypeptide comprises an amino acid sequence selected from the group

consisting of: amino acids 34-91 of SEQ ID NO:2, amino acids 34-92 of SEQ ID NO:4, amino acids 34-99 of SEQ ID NO:6, amino acids 34-92 of SEQ ID NO:8, amino acids 34-93 of SEQ ID NO:10, amino acids 34-91 of SEQ ID NO:12, amino acids 34-91 of SEQ ID NO:14, amino acids 34-92 of SEQ ID NO:16, amino acids 34-91 of SEQ ID NO:18, and amino acids 34-91 of SEQ ID NO:22. In some most preferred embodiments the ADAM disintegrin domain polypeptide comprises an amino acid sequence selected from the group consisting of: amino acids 78-91 of SEQ ID NO:2, amino acids 79-92 of SEQ ID NO:4, amino acids 87-99 of SEQ ID NO:6, amino acids 79-92 of SEQ ID NO:8, amino acids 79-93 of SEQ ID NO:10, amino acids 78-91 of SEQ ID NO:12, amino acids 78-91 of SEQ ID NO:14, amino acids 79-92 of SEQ ID NO:16, amino acids 78-91 of SEQ ID NO:18, and amino acids 78-91 of SEQ ID NO:22.

In some embodiments a therapeutically effective amount of the ADAM disintegrin domain is administered to a mammal in need of such treatment. In preferred embodiments the mammal is afflicted with a condition mediated by angiogenesis, an ocular disorder, malignant or metastatic condition, inflammatory disease, osteoporosis and other conditions mediated by accelerated bone resorption, restenosis, inappropriate platelet activation, recruitment, or aggregation, thrombosis, or a condition requiring tissue repair or wound healing. The ADAM disintegrin domain is, in some embodiments, administered in combination with radiation therapy and/or in combination with one or more additional therapeutic agents.

The invention also encompasses methods for identifying compounds that modulate integrin biological activity, that modulate the interaction between an integrin and an ADAM disintegrin domain, that inhibit endothelial cell migration, or that inhibit angiogenesis, comprising combining a test compound with an integrin or with endothelial cells and with an ADAM disintegrin domain polypeptide that binds to the integrin or endothelial cells and determining whether the test compound alters the binding of the ADAM disintegrin domain polypeptide to the integrin or endothelial cells.

These and other aspects of the present invention will become evident upon reference to the following detailed description, examples, and claims.

DETAILED DESCRIPTION OF THE INVENTION

A. Abbreviations and Terminology Used in the Specification

"4-1BB" and "4-1BB ligand" (4-1BB-L) are polypeptides described, inter alia, in U.S. Patent No. 5,674,704, including soluble forms thereof.

"ADAMs" are a family of transmembrane glycoproteins having disintegrin and metalloproteinase domains, also called MDC, metalloprotease/disintegrin/cysteine-rich proteins.

"Dis" is a disintegrin domain; "ADAMdis" is an ADAM disintegrin domain.

"CD40 ligand" (CD40L) is a polypeptide described, inter alia, in U.S. Patent No. 5,716,805, including soluble forms thereof.

"CD148" is a protein tyrosine phosphatase, also called DEP-1, ECRTF, and PTPRJ. CD148 binding proteins are described in Daniel et al., PCT Publication No. WO 00/15258, 23 March 2000.

"DMEM" is Dulbecco's Modified Eagle Medium.

"FACS" is fluorescence activated cell sorting.

5 "Flt3L" is Flt3 ligand, a polypeptide described, inter alia, in U.S. Patent No. 5,554,512, including soluble forms thereof.

"HRMEC" are human renal microvascular endothelial cells.

"HMVEC-d" are human dermal microvascular endothelial cells.

"mAb" is a monoclonal antibody.

10 "MDC" is a family of cysteine-rich proteins having metalloprotease and disintegrin domains, also called ADAM.

"Nectin-3" is a cell adhesion molecule in the nectin family (which is described, inter alia, in Satoh-Horikawa et al., J. Biol. Chem. 275(14):10291, 2000). The GenBank accession numbers of human nectin-3 nucleic acid and polypeptide sequences are AF282874 and AAF97597 respectively
15 (Reymond et al., 2000).

"PMA" is phorbol-12-myristate-13-acetate.

"Tek," which has also been called Tie2 and ork, is an receptor tyrosine kinase (RTK) that is predominantly expressed in vascular endothelium. The molecular cloning of human Tek (ork) has been described by Ziegler, U.S. Patent No. 5,447,860. "Tek antagonists" are described, inter alia, in
20 Cerretti et al., PCT Publication No. WO 00/75323, 14 December 2000.

"TNF" is tumor necrosis factor. "TNFR" is a tumor necrosis factor receptor, including soluble forms thereof. "TNFR/Fc" is a tumor necrosis factor receptor-Fc fusion polypeptide.

"TRAIL" is TNF-related apoptosis-inducing ligand, a type II transmembrane polypeptide in the TNF family described, inter alia, in U.S. Patent No. 5,763,223, including soluble forms thereof.

25 "TWEAK" is TNF-weak effector of apoptosis, a type II transmembrane polypeptide in the TNF family described, inter alia, in Chicheportiche et al., J. Biol. Chem., 272(51):32401, 1997, including soluble forms thereof. "TWEAK-R" is the "TWEAK receptor," which is described, inter alia, in U.S. Serial Numbers 60/172,878 and 60/203,347 and Feng et al., Am. J. Pathol. 156(4):1253, 2000, including soluble forms thereof. TWEAK-R/Fc is a TWEAK receptor-Fc fusion polypeptide.

30 "VEGF" is vascular endothelial growth factor, also known as VPF or vascular permeability factor.

B. ADAM Polypeptides and ADAM Disintegrin Domain Polypeptides

35 At least thirty ADAMs have been described. Table 1 provides reference information for selected human ADAMs.

ADAM disintegrin domains show sequence homology to the snake venom disintegrins, and are characterized by a framework of cysteines. For example, a typical disintegrin sequence comprises a framework such as:

CDCGX_{3,5}CX_{3,6}CCX_{2,4}CX₇CX_{4,6}CCX_{2,4}CX₈CX_{5,7}CX_{3,5}C (SEQ ID NO:20)

The sequences of several ADAM disintegrin domains are shown in Table 2 and in the Sequence Listing.

- 5 The present invention encompasses the use of various forms of ADAM disintegrin domains that retain at least one activity selected from the group consisting of integrin binding activity, inhibition of endothelial cell migration, and inhibition of angiogenesis. The term "ADAM disintegrin domain polypeptide" is intended to encompass polypeptides containing all or part of a native ADAM disintegrin domain, with or without other ADAM domains (such as the cysteine-rich region), as well
- 10 as related forms including, but not limited to: (a) fragments, (b) variants, (c) derivatives, (d) fusion polypeptides, and (e) multimeric forms (multimers). The ability of these related forms to inhibit integrin binding, endothelial cell migration, and/or inhibition of angiogenesis may be determined in vitro or in vivo by using methods such as those exemplified below or by using other assays known in the art.

15

Table 1
Selected Members of the ADAM Family

ADAM	Other Names	GenBank Accession Number (Human)	Published Description
ADAM-8	MS2, CD156	D26579	Genomics 41(1):56, 1997
ADAM-9	MDC9, meltrin gamma	U41766	J. Cell. Biol. 132(4):717, 1996
ADAM-10	MADM, kuzbanian, reprolysin	AF009615	J. Biol. Chem. 272(39):24588, 1997
ADAM-15	Metargidin, MDC15	U46005	J. Biol. Chem. 271(9):4593, 1996
ADAM-17	TACE, cSVP	U86755	WO 96/41624
ADAM-20	SVPH1-26	AF029899	WO 99/23228
ADAM-21	SVPH1-8	AF029900	WO 99/36549
ADAM-22	SVPH3-13, MDC2	AB009671	WO 99/41388
ADAM-23	SVPH3-17, MDC3	AB009672	WO 99/41388
ADAM-29	SVPH1	AF171929	Biochem. Biophys. Res. Commun. 263:810, 1999

The term "variant" includes polypeptides that are substantially homologous to native ADAM disintegrin domains, but which have an amino acid sequence different from that of a native ADAM disintegrin domain because of one or more deletions, insertions or substitutions. Particular embodiments include, but are not limited to, ADAM disintegrin domain polypeptides that comprise from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native ADAM disintegrin domain sequence. Included as variants of ADAM disintegrin domain polypeptides are those variants that are naturally occurring, such as allelic forms and alternatively spliced forms, as well as variants that have been constructed by modifying the amino acid sequence of a ADAM disintegrin domain polypeptide or the nucleotide sequence of a nucleic acid encoding a ADAM disintegrin domain polypeptide.

Generally, substitutions for one or more amino acids present in the native polypeptide should be made conservatively. Examples of conservative substitutions include substitution of amino acids outside of the active domain(s), and substitution of amino acids that do not alter the secondary and/or tertiary structure of the ADAM disintegrin domain. Additional examples include substituting one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn, or substitutions of one aromatic residue for another, such as Phe, Trp, or Tyr for one another. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are known in the art.

In some preferred embodiments the ADAM disintegrin domain variant is at least about 70% identical in amino acid sequence to the amino acid sequence of a native ADAM disintegrin domain; in some preferred embodiments the ADAM disintegrin domain variant is at least about 80% identical in amino acid sequence to the amino acid sequence of a native ADAM disintegrin domain. In some more preferred embodiments the ADAM disintegrin domain variant is at least about 90% identical in amino acid sequence to the amino acid sequence of a native ADAM disintegrin domain; in some more preferred embodiments the ADAM disintegrin domain variant is at least about 95% identical in amino acid sequence to the amino acid sequence of a native ADAM disintegrin domain. In some most preferred embodiments the ADAM disintegrin domain variant is at least about 98% identical in amino acid sequence to the amino acid sequence of a native ADAM disintegrin domain; in some most preferred embodiments the ADAM disintegrin domain variant is at least about 99% identical in amino acid sequence to the amino acid sequence of a native ADAM disintegrin domain.

Percent identity, in the case of both polypeptides and nucleic acids, may be determined by visual inspection. Percent identity may be determined using the alignment method of Needleman and Wunsch (J. Mol. Biol. 48:443, 1970) as revised by Smith and Waterman (Adv. Appl. Math 2:482, 1981). Preferably, percent identity is determined by using a computer program, for example, the GAP computer program version 10.x available from the Genetics Computer Group (GCG; Madison, WI, see also Devereux et al., *Nucl. Acids Res.* 12:387, 1984). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-

identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979 for amino acids; (2) a
5 (nucleotides) penalty for each symbol in each gap; (3) no penalty for end gaps; and (4) no maximum
penalty for long gaps. Other programs used by one skilled in the art of sequence comparison may also
be used. For fragments of ADAM disintegrin domains, the percent identity is calculated based on that
portion of ADAM disintegrin domain that is present in the fragment.

When a deletion or insertion strategy is adopted, the potential effect of the deletion or
10 insertion on biological activity (such as integrin binding activity, inhibition of endothelial cell
migration, or inhibition of angiogenesis) must be considered. Subunits of the inventive polypeptides
may be constructed by deleting terminal or internal residues or sequences. Additional guidance as to
the types of mutations that can be made is provided by a comparison of the sequence of ADAM
disintegrin domain polypeptides to polypeptides that have similar structures, as well as by performing
15 structural analysis of the inventive polypeptides.

The term "variant" also includes ADAM disintegrin domain polypeptides that are encoded by
nucleic acids capable of hybridizing under moderately stringent conditions (e.g., prewashing solution
of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50°C, 5 X SSC,
overnight) or higher stringency conditions to DNA sequences encoding ADAM disintegrin domain
20 polypeptides, and which encode polypeptides that retain at least one activity selected from the group
consisting of integrin binding activity, inhibition of endothelial cell migration, and inhibition of
angiogenesis. The skilled artisan can determine additional combinations of salt and temperature that
constitute moderate hybridization stringency. Conditions of higher stringency include higher
temperatures for hybridization and post-hybridization washes, and/or lower salt concentration.

25 Mutations can be introduced into nucleic acids by synthesizing oligonucleotides containing a
mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence.
Following ligation, the resulting reconstructed sequence encodes a variant having the desired amino
acid insertion, substitution, or deletion. Alternatively, oligonucleotide-directed site-specific
mutagenesis procedures can be employed to provide an altered gene having particular codons altered
30 according to the substitution, deletion, or insertion required. The well known polymerase chain
reaction (PCR) procedure also may be employed to generate and amplify a DNA sequence encoding a
desired polypeptide or fragment thereof. Oligonucleotides that define the desired termini of the DNA
fragment are employed as 5' and 3' primers. The oligonucleotides may additionally contain
recognition sites for restriction endonucleases to facilitate insertion of the amplified DNA fragment
35 into an expression vector.

The present invention further encompasses the use of ADAM disintegrin domain polypeptides
with or without associated native-pattern glycosylation. ADAM disintegrin domain expressed in yeast
or mammalian expression systems (e.g., COS-1 or COS-7 cells) may be similar to or significantly

different from a native ADAM disintegrin domain polypeptide in molecular weight and glycosylation pattern, depending upon the choice of expression system. Expression of ADAM disintegrin domain polypeptides in bacterial expression systems, such as *E. coli*, provides non-glycosylated molecules. Different host cells may also process polypeptides differentially, resulting in heterogeneous mixtures of polypeptides with variable N- or C-termini.

The primary amino acid structure of ADAM disintegrin domain polypeptides may be modified to create derivatives by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives of ADAM disintegrin domain polypeptides may be prepared by linking particular functional groups to ADAM disintegrin domain amino acid side chains or at the N-terminus or C-terminus of a ADAM disintegrin domain polypeptide.

Fusion polypeptides of ADAM disintegrin domains that are useful in practicing the invention include covalent or aggregative conjugates of ADAMdis or its fragments with other polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. One class of fusion polypeptides are discussed below in connection with ADAM disintegrin oligomers. As another example, a fusion polypeptide may comprise a signal peptide (which is also variously referred to as a signal sequence, signal, leader peptide, leader sequence, or leader) at the N-terminal region or C-terminal region of an ADAM disintegrin domain polypeptide which co-translationally or post-translationally directs transfer of the polypeptide from its site of synthesis to a site inside or outside of the cell membrane or cell wall. It is particularly advantageous to fuse a signal peptide that promotes extracellular secretion to the N-terminus of a soluble ADAMdis polypeptide. In this case, the signal peptide is typically cleaved upon secretion of the soluble polypeptide from the cell.

Secreted soluble polypeptides may be identified (and distinguished from its non-soluble membrane-bound counterparts) by separating intact cells which express the desired polypeptide from the culture medium, e.g., by centrifugation, and assaying the medium (supernatant) for the presence of the desired polypeptide. The presence of the desired polypeptide in the medium indicates that the polypeptide was secreted from the cells and thus is a soluble form of the polypeptide. Soluble polypeptides may be prepared by any of a number of conventional techniques. A DNA sequence encoding a desired soluble polypeptide may be subcloned into an expression vector for production of the polypeptide, or the desired encoding DNA fragment may be chemically synthesized.

Soluble ADAM disintegrin domain polypeptides comprise all or part of the ADAM disintegrin domain, with or without additional segments from the extracellular portion of the ADAM (such as the cysteine-rich region) but generally lack a transmembrane domain that would cause retention of the polypeptide at the cell surface. Soluble polypeptides may include part of the transmembrane domain or all or part of the cytoplasmic domain as long as the polypeptide is secreted from the cell in which it is produced. Examples of soluble ADAM disintegrin domain polypeptides are provided in the examples. In some preferred embodiments of the present invention, a multimeric form of a soluble ADAM disintegrin domain polypeptide is used to inhibit integrin binding to ligands

and, hence, integrin biological activity. In some most preferred embodiments the soluble ADAM disintegrin domain polypeptide is used to inhibit endothelial cell migration and/or inhibit angiogenesis. These inhibitory activities may include both integrin-mediated and integrin-independent mechanisms.

ADAM disintegrin domain multimers are covalently-linked or non-covalently-linked
5 multimers, including dimers, trimers, and higher multimers. Oligomers may be linked by disulfide bonds formed between cysteine residues on different ADAM disintegrin domain polypeptides. One embodiment of the invention is directed to multimers comprising multiple ADAM disintegrin domain polypeptides joined via covalent or non-covalent interactions between peptide moieties fused to the ADAM disintegrin domain polypeptides. Such peptides may be peptide linkers (spacers), or peptides
10 that have the property of promoting multimerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote multimerization of ADAM disintegrin domain polypeptides attached thereto, as described in more detail below. In particular embodiments, the multimers comprise from two to four ADAM disintegrin domain polypeptides.

In some embodiments, a ADAM disintegrin domain multimer is prepared using polypeptides
15 derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, e.g., by Ashkenazi et al. (Proc. Natl. Acad. Sci. USA 88:10535, 1991); Byrn et al. (Nature 344:677, 1990); and Hollenbaugh and Aruffo ("Construction of Immunoglobulin Fusion Proteins", in Current Protocols in Immunology, Suppl. 4, pages 10.19.1-10.19.11, 1992).

20 A preferred embodiment of the present invention is directed to an ADAM disintegrin domain (ADAMdis) dimer comprising two fusion polypeptides created by fusing an ADAM disintegrin domain to an Fc polypeptide. A gene fusion encoding the ADAMdis-Fc fusion polypeptide is inserted into an appropriate expression vector. ADAMdis-Fc fusion polypeptides are expressed in host cells transformed with the recombinant expression vector, and allowed to assemble much like antibody
25 molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield divalent soluble ADAMdis polypeptides. The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides derived from the Fc region of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization are also included.

One suitable Fc polypeptide, described in PCT application WO 93/10151, is a single chain
30 polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and by Baum et al., EMBO J. 13:3992, 1994. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22
35 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors. Fusion polypeptides comprising Fc moieties, and multimers formed therefrom, offer an advantage of facile purification by affinity chromatography over Protein A or Protein G columns, and Fc fusion

polypeptides may provide a longer in vivo half life, which is useful in therapeutic applications, than unmodified polypeptides.

5 In other embodiments, a soluble ADAM disintegrin domain polypeptide may be substituted for the variable portion of an antibody heavy or light chain. If fusion proteins are made with both heavy and light chains of an antibody, it is possible to form an ADAM disintegrin domain multimer with as many as four soluble ADAM disintegrin domain polypeptides.

Alternatively, the ADAM disintegrin domain multimer is a fusion polypeptide comprising multiple ADAM disintegrin domain polypeptides, with or without peptide linkers (spacers), or peptides that have the property of promoting multimerization.. Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233. A DNA sequence encoding a desired peptide linker may be inserted between, and in the same reading frame as, the DNA sequences encoding ADAMdis, using conventional techniques known in the art. For example, a chemically synthesized oligonucleotide encoding the linker may be ligated between sequences encoding ADAMdis. In particular embodiments, a fusion protein comprises from two to four ADAM

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disintegrin domain polypeptides, separated by peptide linkers.

Another method for preparing ADAM disintegrin domain multimers involves use of a leucine zipper domain. Leucine zipper domains are peptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, 1988), and have since been found in a variety of different

20 proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe et al. FEBS Lett. 344:191, 1994. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow et al., Semin. Immunol. 6:267, 1994. Recombinant fusion polypeptides comprising an ADAM disintegrin domain polypeptide fused to a leucine zipper peptide are expressed in suitable host cells, and the ADAM disintegrin domain multimer that forms is recovered from the culture supernatant.

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30 C. Recombinant Production of ADAM Disintegrin Domain Polypeptides

The ADAM disintegrin domain polypeptides used in the present invention may be prepared using a recombinant expression system. Host cells transformed with a recombinant expression vector encoding the ADAM disintegrin domain polypeptide are cultured under conditions that promote expression of ADAM disintegrin domain and the ADAM disintegrin domain is recovered. ADAM

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disintegrin domain polypeptides can also be produced in transgenic plants or animals.

Any suitable expression system may be employed. Recombinant expression vectors include DNA encoding an ADAM disintegrin domain polypeptide operably linked to suitable transcriptional

and translational regulatory nucleotide sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the ADAM disintegrin domain DNA sequence. Thus, a promoter nucleotide sequence is operably linked to an ADAM disintegrin domain DNA sequence if the promoter
5 nucleotide sequence controls the transcription of the ADAM disintegrin domain DNA sequence. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, an mRNA ribosomal binding site, and appropriate sequences which control transcription and translation initiation and termination. A sequence encoding an appropriate signal peptide (native or heterologous) can be incorporated into expression vectors. A DNA sequence for a signal peptide (secretory leader)
10 may be fused in frame to the ADAM disintegrin domain sequence so that the ADAM disintegrin domain polypeptide is initially translated as a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cells promotes extracellular secretion of the ADAM disintegrin domain polypeptide. The signal peptide is cleaved from the ADAM disintegrin domain polypeptide upon secretion from the cell. Suitable host cells for expression of ADAM disintegrin
15 domain polypeptides include prokaryotes, yeast and higher eukaryotic cells, including insect and mammalian cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, insect, and mammalian cellular hosts are known in the art.

Using the techniques of recombinant DNA including mutagenesis and the polymerase chain reaction (PCR), the skilled artisan can produce DNA sequences that encode ADAM disintegrin
20 domain polypeptides comprising various additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences, including ADAM disintegrin domain fragments, variants, derivatives, multimers, and fusion polypeptides.

The procedures for purifying expressed ADAM disintegrin domain polypeptides will vary according to the host system employed, and whether or not the recombinant polypeptide is secreted.
25 ADAM disintegrin domain polypeptides may be purified using methods known in the art, including one or more concentration, salting-out, ion exchange, hydrophobic interaction, affinity purification, HPLC, or size exclusion chromatography steps. Fusion polypeptides comprising Fc moieties (and multimers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

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D. Therapeutic Methods

The disclosed methods may be used to inhibit integrin binding and integrin biological activity, and to inhibit endothelial cell migration, and/or angiogenesis in a mammal in need of such treatment. The treatment is advantageously administered in order to prevent the onset or the recurrence of a
35 disease or condition mediated by an integrin, or to treat a mammal that has a disease or condition mediated by an integrin.

Examples of the therapeutic uses of ADAM disintegrin domain polypeptides and compositions thereof include the treatment of individuals afflicted with conditions mediated by

angiogenesis such as ocular disorders, dermatological disorders, and malignant or metastatic conditions, inflammatory diseases, osteoporosis and other conditions mediated by accelerated bone resorption, restenosis, inappropriate platelet activation, recruitment, or aggregation, thrombosis, or a condition requiring tissue repair or wound healing.

5 Among the ocular disorders that can be treated according to the present invention are eye diseases characterized by ocular neovascularization including, but not limited to, diabetic retinopathy (a major complication of diabetes), retinopathy of prematurity (this devastating eye condition, that frequently leads to chronic vision problems and carries a high risk of blindness, is a severe complication during the care of premature infants), neovascular glaucoma, retinoblastoma, retrolental
10 fibroplasia, rubeosis, uveitis, macular degeneration, and corneal graft neovascularization. Other eye inflammatory diseases, ocular tumors, and diseases associated with choroidal or iris neovascularization can also be treated according to the present invention.

 The present invention can also be used to treat malignant and metastatic conditions such as solid tumors. Solid tumors include both primary and metastatic sarcomas and carcinomas.

15 The present invention can also be used to treat inflammatory diseases including, but not limited to, arthritis, rheumatism, inflammatory bowel disease, and psoriasis.

 Among the conditions mediated by inappropriate platelet activation, recruitment, aggregation, or thrombosis that can be treated according to the present invention are coronary artery disease or injury, myocardial infarction or injury following myocardial infarction, stroke, unstable angina,
20 atherosclerosis, arteriosclerosis, preeclampsia, embolism, platelet-associated ischemic disorders including lung ischemia, coronary ischemia, and cerebral ischemia, restenosis following percutaneous coronary intervention including angioplasty, atherectomy, stent placement, and bypass surgery, thrombotic disorders including coronary artery thrombosis, cerebral artery thrombosis, intracardiac thrombosis, peripheral artery thrombosis, venous thrombosis, thrombosis and coagulopathies
25 associated with exposure to a foreign or injured tissue surface, and reocclusion following thrombosis, deep venous thrombosis (DVT), pulmonary embolism (PE), transient ischemic attacks (TIAs), and another conditions where vascular occlusion is a common underlying feature. In some embodiments the methods according to the invention are used in individuals at high risk for thrombus formation or reformation, advanced coronary artery disease, or for occlusion, reocclusion, stenosis and/or restenosis
30 of blood vessels, or stroke. In some embodiments the methods according to the invention are used in combination with angioplasty procedures, such as balloon angioplasty, laser angioplasty, coronary atherectomy or similar techniques, carotid endarterectomy, anastomosis of vascular grafts, surgery having a high risk of thrombus formation (i.e., coronary bypass surgery, insertion of a prosthetic valve or vessel and the like), atherectomy, stent placement, placement of a chronic cardiovascular device
35 such as an in-dwelling catheter or prosthetic valve or vessel, organ transplantation, or bypass surgery.

 Other diseases and conditions that can be treated according to the present invention include benign tumors and preneoplastic conditions, myocardial angiogenesis, hemophilic joints, scleroderma,

vascular adhesions, asthma and allergy, eczema and dermatitis, graft versus host disease, sepsis, adult respirator distress syndrome, telangiectasia, and wound granulation.

5 The methods according to the present invention can be tested in in vivo animal models for the desired prophylactic or therapeutic activity, as well as to determine the optimal therapeutic dosage, prior to administration to humans.

The amount of a particular ADAM disintegrin domain polypeptide that will be effective in a particular method of treatment depends upon age, type and severity of the condition to be treated, body weight, desired duration of treatment, method of administration, and other parameters. Effective dosages are determined by a physician or other qualified medical professional. Typical effective
10 dosages are about 0.01 mg/kg to about 100 mg/kg body weight. In some preferred embodiments the dosage is about 0.1-50 mg/kg; in some preferred embodiments the dosage is about 0.5-10 mg/kg. The dosage for local administration is typically lower than for systemic administration. In some embodiments a single administration is sufficient; in some embodiments the ADAM disintegrin domain is administered as multiple doses over one or more days.

15 The ADAM disintegrin domain polypeptides are typically administered in the form of a pharmaceutical composition comprising one or more pharmacologically acceptable carriers. Pharmaceutically acceptable carriers include diluents, fillers, adjuvants, excipients, and vehicles which are pharmaceutically acceptable for the route of administration, and may be aqueous or oleaginous suspensions formulated using suitable dispersing, wetting, and suspending agents.

20 Pharmaceutically acceptable carriers are generally sterile and free of pyrogenic agents, and may include water, oils, solvents, salts, sugars and other carbohydrates, emulsifying agents, buffering agents, antimicrobial agents, and chelating agents. The particular pharmaceutically acceptable carrier and the ratio of active compound to carrier are determined by the solubility and chemical properties of the composition, the mode of administration, and standard pharmaceutical practice.

25 The ADAM disintegrin domain polypeptides are administered to the patient in a manner appropriate to the indication. Thus, for example, ADAM disintegrin domain polypeptides, or pharmaceutical compositions thereof, may be administered by intravenous, transdermal, intradermal, intraperitoneal, intramuscular, intranasal, epidural, oral, topical, subcutaneous, intracavity, sustained release from implants, peristaltic routes, or by any other suitable technique. Parenteral administration
30 is preferred.

In certain embodiments of the claimed invention, the treatment further comprises treating the mammal with one or more additional therapeutic agents. The additional therapeutic agent(s) may be administered prior to, concurrently with, or following the administration of the ADAM disintegrin domain polypeptide. The use of more than one therapeutic agent is particularly advantageous when
35 the mammal that is being treated has a solid tumor. In some embodiments of the claimed invention, the treatment further comprises treating the mammal with radiation. Radiation, including brachytherapy and teletherapy, may be administered prior to, concurrently with, or following the administration of the ADAM disintegrin domain polypeptide and/or additional therapeutic agent(s).

In some preferred embodiments the method includes the administration of, in addition to an ADAM disintegrin domain polypeptide, one or more therapeutics selected from the group consisting of alkylating agents, antimetabolites, vinca alkaloids and other plant-derived chemotherapeutics, antitumor antibiotics, antitumor enzymes, topoisomerase inhibitors, platinum analogs, adrenocortical
5 suppressants, hormones and antihormones, antibodies, immunotherapeutics, radiotherapeutics, and biological response modifiers.

In some preferred embodiments the method includes administration of, in addition to an ADAM disintegrin domain polypeptide, one or more therapeutics selected from the group consisting of cisplatin, cyclophosphamide, mechlorethamine, melphalan, bleomycin, carboplatin, fluorouracil, 5-
10 fluorodeoxyuridine, methotrexate, taxol, asparaginase, vincristine, and vinblastine, lymphokines and cytokines such as interleukins, interferons (alpha., beta. or delta.) and TNF, chlorambucil, busulfan, carmustine, lomustine, semustine, streptozocin, dacarbazine, cytarabine, mercaptopurine, thioguanine, vindesine, etoposide, teniposide, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, mitomycin, L-asparaginase, hydroxyurea, methylhydrazine, mitotane, tamoxifen, fluoxymesterone,
15 IL-8 inhibitors, angiostatin, endostatin, krigle 5, angiopoietin-2 or other antagonists of angiopoietin-1, antagonists of platelet-activating factor, antagonists of basic fibroblast growth factor, and COX-2 inhibitors.

In some preferred embodiments the method includes administration of, in addition to an ADAM disintegrin domain polypeptide, one or more therapeutic polypeptides, including soluble forms
20 thereof, selected from the group consisting of Flt3 ligand, CD40 ligand, interleukin-2, interleukin-12, 4-1BB ligand, anti-4-1BB antibodies, TRAIL, TNF antagonists and TNF receptor antagonists including TNFR/Fc, Tek antagonists, TWEAK antagonists and TWEAK-R antagonists including TWEAK-R/Fc, VEGF antagonists including anti-VEGF antibodies, VEGF receptor (including VEGF-R1 and VEGF-R2, also known as Flt1 and Flk1 or KDR) antagonists, CD148 (also referred to as DEP-
25 1, EC RTP, and PTPRJ, see Takahashi et al., J. Am. Soc. Nephrol. 10:2135-45, 1999; and PCT Publication No. WO 00/15258, 23 March 2000) binding proteins, and nectin-3 antagonists.

In some preferred embodiments the ADAM disintegrin domain polypeptides of the invention are used as a component of, or in combination with, "metronomic therapy," such as that described by Browder et al. and Klement et al. (Cancer Research.60:1878, 2000; J. Clin. Invest. 105(8):R15, 2000;
30 see also Barinaga, Science 288:245, 2000).

As used herein, the terms "therapy," "therapeutic," "treat," and "treatment" generally include prophylaxis, i.e. prevention, in addition to therapy or treatment for an extant disease or condition. The methods of the present invention may be used as a first line treatment, for the treatment of residual disease following primary therapy, or as an adjunct to other therapies. Methods of measuring
35 biological effectiveness are known in the art and are illustrated in the Examples below.

EXAMPLES

The following examples are intended to illustrate particular embodiments and not to limit the scope of the invention.

EXAMPLE 1

ADAM Disintegrin Domain Polypeptides

This example describes one method for the recombinant production of ADAM disintegrin domain polypeptides.

Expression cassettes encoding an IgKappa leader sequence, ADAM disintegrin domain, and C-terminal Fc region were constructed in bacterial plasmids then transferred into eukaryotic expression vectors (pDC409, EMBO J. 10:2821, 1991, or another mammalian expression vector). The coding regions of the various constructs are summarized in Table 2. In addition to the disintegrin domain, these constructs encode additional portions of the extracellular portion of the ADAM (e.g., cysteine-rich region and EGF-like domain).

The expression vectors were transfected into COS-1, CV-1/EBNA, or 293/EBNA cells. Two days after transfection the cells were ^{35}S labeled for four hours. Supernatants and total cell lysates were prepared and aliquots were immunoprecipitated using protein A-sepharose beads to capture the Fc tagged polypeptides. ^{35}S labeled ADAM disintegrin-Fc polypeptides were run on 8-16% reducing gels and detected via autoradiography.

The cell type that produced the most soluble protein in the supernatant was used in a large scale (T-175 format, 20 flasks) transient transfection, and approximately one liter of supernatant was harvested after one week. ADAM disintegrin-Fc polypeptides were purified from the supernatants using affinity chromatography (protein A column). The polypeptides were characterized by determining the N-terminal amino acid sequence, amino acid composition, and protein integrity (SDS-PAGE under reducing and non-reducing conditions) before the polypeptides were used in FACS, immunoprecipitations, and biological assays such as those described below.

Table 2
ADAM Disintegrin Domain Polypeptide Constructs

Construct	SEQ ID NOs: DNA/polypeptide	IgK Leader ^{1,2}	ADAM disintegrin ^{1,3} (dis Framework) ^{1,4}	Fc Region ¹
ADAM-8dis-Fc	1/2	1-20	23-264 (34-91)	267-494
ADAM-9dis-Fc	3/4	1-20	23-303 (34-92)	306-533
ADAM-10dis-Fc	5/6	1-20	23-235 (34-99)	238-465
ADAM-15dis-Fc	7/8	1-20	23-292 (34-92)	295-522
ADAM-17dis-Fc	9/10	1-20	23-216 (34-93)	219-446
ADAM-20dis-Fc	11/12	1-20	23-305 (34-91)	308-535
ADAM-21dis-Fc	13/14	1-20	23-293 (34-91)	296-523
ADAM-22dis-Fc	15/16	1-20	23-312 (34-92)	315-542
ADAM-23dis-Fc	17/18	1-20	23-310 (34-91)	313-540
ADAM-29dis-Fc	21/22	1-20	23-298 (34-91)	301-528

¹ residues in the polypeptide sequence

5 ² the predicted cleavage site is after residue 20

³ segment of the construct that includes ADAMdis, but may also contain additional ADAM sequences

⁴ disintegrin framework, e.g., SEQ ID NO:20

EXAMPLE 2

10 **Binding of ADAM Disintegrin Domain Polypeptides to Cells**

A. Binding to Endothelial cells

This example describes a flow cytometric integrin mAb based binding inhibition assay, which is used to show binding of ADAM disintegrin-Fc polypeptides to integrins expressed on the surface of endothelial cells. Human endothelial cells express $\alpha\beta_1$, $\alpha\beta_3$, β_1 , β_3 , α_1 , α_2 , α_3 , α_4 , α_5 , and α_v integrins.

15 Primary human dermal microvascular endothelial cells (HMVEC-d) were maintained in supplemented endothelial growth medium (Clonetics Corporation, Walkersville, MD). The ADAM disintegrin-Fc polypeptides produced in Example 1 were shown to bind specifically to HMVEC-d.

Monoclonal antibodies specific for human integrins $\alpha_v\beta_3$ (LM609, anti CD51/61, Chemicon, Temecula, CA Brooks et al., Science 264:569, 1994), $\alpha_2\beta_1$ (BHA2.1 anti CD49b, Chemicon, Wang et al., Mol. Biol. of the Cell 9:865, 1998), $\alpha_5\beta_1$ (SAM-1 anti CD49e, Biodesign, A. te Velde et al., J. Immunol. 140:1548, 1988), $\alpha_3\beta_1$ (ASC-6 anti-CD49c, Chemicon, Pattaramalai et al., Exp. Cell. Res. 222: 281, 1996), $\alpha_4\beta_1$ (HP2/1 anti CD49d, Immunotech, Marseilles, France. Workshop of the 4th International Conference on Human Leukocyte Differentiation Antigens, Vienna Austria, 1989, workshop number p091), $\alpha_6\beta_1$ (GoH3 anti CD49f, Immunotech, Workshop 4th International Conference on Human Leukocyte Differentiation Antigens, workshop number p055), $\alpha_6\beta_4$ (439-9B anti CD104, Pharmingen, San Diego, CA., Schlossman et al., 1995 Leukocyte Typing V: White Cell Differentiation Antigens. Oxford University Press, New York), and $\alpha_v\beta_5$ (MAB 1961, Chemicon International, monoclonal anti-human integrin $\alpha_v\beta_5$ mAb, IgG1 isotype, inhibits $\alpha_v\beta_5$ mediated binding/adhesion to vitronectin/fibronectin; Weinaker, et al., J. Biol. Chem. 269:6940, 1994) were also shown to bind specifically to HMVEC-d. Each of these antibodies is known to specifically block binding of the indicated integrin to its ligands (e.g., fibronectin, vitronectin, fibrinogen). The ability of integrin mAbs to inhibit the binding of ADAM disintegrin-Fc polypeptides reveals which integrins the disintegrin domains bind and, indirectly, which integrin binding activities the disintegrin domains are able to antagonize. The ability of the antibodies to inhibit binding of the ADAM disintegrin-Fc polypeptides to endothelial cells was tested as described below.

Prior to performing binding studies, HMVEC-d were removed from culture vessels using trypsin-EDTA. The cells were washed in media containing serum and resuspended in binding medium which consisted of PBS containing 1 mM Ca²⁺, 1 mM Mg²⁺ and 0.5 mM Mn²⁺, 0.1% sodium azide, 10% Normal goat serum, 2% rabbit serum and 2% fetal bovine serum. Under these binding conditions, ADAM-8, -9, -10, -15, -17, -20, -21, -22, -23, and -29dis-Fc all bind to human endothelial cells.

One hundred microliters of cell suspension, containing 200,000 to 500,000 HMVEC-d, were added to 12x75mm plastic test tubes. Monoclonal antibodies specific for one of the integrins, or a control monoclonal antibody (CD29 or M15), were added to the cell suspensions at a concentration of 100 μ g/ml (5-8 fold mass excess) 15 minutes prior to addition of disintegrin-Fc fusion proteins. ADAM disintegrin-Fc polypeptides and control Fc fusion polypeptides (P7.5II.Fc) were added, at various concentrations from 12.5 to 20 μ g/ml, to the cell suspensions and incubated for 1 hour at 30° C. Unbound Fc polypeptides were washed away by centrifugation of cells in 2 mls of binding media. The washed cell pellets were resuspended in binding medium and then incubated at 30° C for 30 minutes with goat anti-human Fc-specific biotinylated antibody at a concentration of 2.5 μ g/ml for 30 minutes. After centrifugation and washing of the cell pellets, the cells were resuspended in binding medium and bound anti-human Fc-biotin was detected by adding streptavidin-phycoerythrin conjugate to the cell suspension at a 1:1000 dilution (1 μ g/ml) and incubating at 30° C for 30 minutes. The unbound streptavidin-phycoerythrin was washed away and the cells were resuspended in binding

medium containing propidium iodide. The level of fluorescent binding (disintegrin-Fc binding) was determined by flow cytometry.

5 The level of binding of each ADAM disintegrin-Fc polypeptide was determined in the presence of anti-integrin specific mAb and in the presence of control mAb. Both the intensity of binding (MFI) and the percentage of cells binding were determined. Percent inhibition was calculated using the formula $[1 - (\text{MFI control} - \text{MFI integrin mAb}) / \text{MFI control}]$. The results of these studies are summarized in Table 3.

10 ADAM-15, -17, -20 and -22 disintegrin domain polypeptides bound to $\alpha_5\beta_3$; ADAM 23 disintegrin domain polypeptide bound to $\alpha_2\beta_1$; ADAM-15, -21, -22 and -23 disintegrin domain polypeptides bound to $\alpha_5\beta_1$; ADAM-10, -17, -22 and -23 disintegrin domain polypeptides bound to the α_6 integrins; ADAM-10 and -15 disintegrin domain polypeptides bound to $\alpha_v\beta_5$. An excess of a non blocking $\alpha_v\beta_5$ antibody did significantly affect the binding of ADAM-10, -22, and -23 disintegrin polypeptides to endothelial cells, suggesting that these ADAMdis polypeptides interact with integrin sites other than or in addition to the ligand (e.g., fibronectin, vitronectin) binding site. Based upon results from a different type of assay, Cal et al. have reported that the ADAM-23 disintegrin domain interacts with the $\alpha_v\beta_3$ integrin through an RGD-independent mechanism (Molec. Biol. of the Cell 11:1457, 2000).

15 Binding experiments are repeated using other ADAM disintegrin domains and other monoclonal antibodies. ADAM disintegrin-Fc polypeptides that bind to selected integrins are further tested for the ability to disrupt integrin-ligand interactions and to modulate endothelial cell function, angiogenesis, and other biological activities in vitro and in vivo.

Table 3
Binding of ADAM Disintegrin-Fc Polypeptides to Integrins Expressed on Human Endothelial Cells

ADAM	Integrin									
	Binding ¹ (+ or - or ND, not done) and Percent (%) Binding ²									
	$\alpha_v\beta_3$	$\alpha_3\beta_1$	$\alpha_4\beta_1$	$\alpha_5\beta_1$	$\alpha_6\beta_1$	$\alpha_4\beta_1$	$\alpha_5\beta_1$	$\alpha_6\beta_1, \alpha_6\beta_4$	$\alpha_6\beta_5$	
ADAM-8	ND	ND	- (<10)	- (<10)	- (<10)	ND	ND	ND	- (<20)	
ADAM-9	- (<10)	- (<10)	- (<10)	- (<10)	- (<20)	- (<10)	- (<10)	- (<10)	- (<10)	
ADAM-10	- (<10)	- (<10)	- (<10)	- (<10)	- (<20)	- (<10)	- (<10)	+ (48)	+ (25)	
ADAM-15	+ (60)	- (<10)	- (<10)	- (<10)	- (<20)	- (<10)	+ (30)	- (<10)	+ (25)	
ADAM-17	+ (50)	- (<10)	- (<10)	- (<10)	- (<10)	- (<10)	- (<10)	+ (69)	- (<10)	
ADAM-20	+ (58)	- (<10)	- (<10)	- (<10)	- (<10)	- (<10)	- (<20)	- (<10)	- (<10)	
ADAM-21	- (<10)	- (<10)	- (<10)	- (<10)	- (<10)	- (<10)	+ (54)	- (<10)	- (<10)	
ADAM-22	+ (42)	- (<10)	- (<10)	- (<10)	- (<10)	- (<10)	+ (36)	+ (32)	- (<10)	
ADAM-23	- (<10)	+ (22)	- (<10)	- (<10)	- (<10)	- (<10)	+ (49)	+ (31)	- (<10)	

¹ positive binding defined as >20% binding inhibition; normal background variation 5-10%, baseline positive approx. 2X over background

² percent inhibition of binding by ADAM-dis-Fc in the presence of 5-8 fold excess integrin mAb as compared to control mAb

B. Binding to Primary Human T-Cells

Primary human T-cells were purified from whole blood. These cells were used in FACS experiments to assess cell surface binding of purified ADAMdis-Fc polypeptides. ADAMdis-Fc binding was assessed with and without Con A (5 µg/ml) or immobilized OTK3 antibody (1 mg/ml, immobilized for 1 hour, 37°C) stimulation. ADAMdis-Fc polypeptides (20 µg/ml) were bound at either 4° C or 30° C in the presence of cations (Ca++, Mg++, Mn++, 0.5 mM each). Cell surface integrin expression was assessed using a panel of murine and rat anti-human integrin antibodies. α₅, α₁, α₃, α₄, α₆, β₁, and β₇ integrins were detected on the surface of these cells. ADAMdis-Fc polypeptides did not bind to primary human T-cells at 4° C. ADAM-8-, ADAM-9-, ADAM-15-, ADAM-20-, ADAM-21-, ADAM-22-, and ADAM-23-dis-Fc polypeptides did bind primary T-cells at 30° C with Con A stimulation. ADAMdis-Fc binding was not inhibited by a three-fold molar excess of antibodies to the integrins listed above.

C. Binding to Resting Platelets

Binding of ADAMdis-Fc polypeptides to citrated washed resting platelets was performed at 4°C or 30°C. Binding was analyzed by flow cytometry using a biotinylated-anti-human Fc specific antibody and streptavidin-PE. Resting platelets express the integrins CD41/CD61 and CD49e. ADAM-9dis-Fc and ADAM-8dis-Fc bound resting platelets at 30°C but not at 4°C. ADAM-9dis-Fc binding to resting platelets at 30°C was not inhibited by a ten-fold excess of CD41a mAb.

EXAMPLE 3**Activity of ADAM Disintegrin Domain Polypeptides In a Wound Closure Assay**

A planar endothelial cell migration (wound closure) assay was used to quantitate the inhibition of angiogenesis by ADAM disintegrin-Fc polypeptides in vitro. In this assay, endothelial cell migration is measured as the rate of closure of a circular wound in a cultured cell monolayer. The rate of wound closure is linear, and is dynamically regulated by agents that stimulate and inhibit angiogenesis in vivo.

Primary human renal microvascular endothelial cells, HRMEC, were isolated, cultured, and used at the third passage after thawing, as described in Martin et al., In Vitro Cell Dev Biol 33:261, 1997. Replicate circular lesions, "wounds," (600-800 micron diameter) were generated in confluent HRMEC monolayers using a silicon-tipped drill press. At the time of wounding the medium (DMEM + 1% BSA) was supplemented with 20 ng/ml PMA (phorbol-12-myristate-13-acetate), a range of concentrations of ADAM disintegrin-Fc polypeptide, or combinations of PMA and ADAM disintegrin-Fc polypeptide. The residual wound area was measured as a function of time (0-12 hours) using a microscope and image analysis software (Bioquant, Nashville, TN). The relative migration rate was calculated for each agent and combination of agents by linear regression of residual wound

area plotted over time. The inhibition of PMA-induced endothelial migration by ADAM disintegrin-Fc polypeptides is shown in Table 4.

The effect of ADAM-dis-Fc polypeptides on EGF-induced migration was also determined. For these experiments EGF (epidermal growth factor, 40 ng/ml) was added to the medium, instead of PMA, at the time of wounding. The results are shown in Table 5.

Table 4

Effect of ADAM-15, -17, -20, and -23dis-Fc Polypeptides in PMA-Induced Endothelial Cell Wound Closure Migration Assay

Expt. ID	No Addition	PMA 20 ng/ml	PMA + IgG	PMA + ADAM-15dis-Fc	PMA + ADAM-17dis-Fc	PMA + ADAM-20dis-Fc	PMA + ADAM-23dis-Fc
HL-H-142 15 µg/ml dis-Fc	0.0436 ¹ (0.0016) ²	0.0655 (0.0004)				0.0499 (0.0009) 72% ³	
HL-H-147 15 µg/ml dis-Fc	0.0244 (0.0023)	0.0424 (0.0002)	0.0449 (0.0012) 0%	0.0357 (0.0007) 37%			0.0225 (0.0022) 100%
HL-H-153 15 µg/ml dis-Fc	0.0253 0.00013	0.0460 (0.0022)	0.0491 (0.006) 0%		0.0392 (0.0016) 33%	0.0388 (0.005) 36%	0.0317 (0.005) 70%
HL-H-154 15 µg/ml dis-Fc	0.0119 (0.0012)	0.0312 (0.0016)			0.0283 (0.0008) 15%	0.0160 (0.0017) 79%	

¹ Slopes to average triplicate Y values and treat as a single data point in order to test whether the slopes are significantly different

² Data in parentheses is the +/- standard error of slopes

³ Percent inhibition compared to migration rate observed in the presence of PMA

Table 5

Effect of ADAM-17, -20, and -23dis-Fc Polypeptides in EGF-Induced Endothelial Cell Wound Closure Migration Assay

Expt. ID	No Addition	EGF 40 ng/ml	EGF + IgG	EGF + ADAM-17dis-Fc	EGF + ADAM-20dis-Fc	EGF + ADAM-23dis-Fc
HL-H-154 15 µg/ml dis-Fc	0.0119 (0.0012)	0.0378 (0.0061)		0.0242 (0.0029) 53%	0.0172 (0.0031) 80%	0.0310 (0.0036) 26%
HL-H-155 9 µg/ml dis-Fc	0.0164 (0.0010)	0.0468 (0.0059)	0.0454 (0.0052) 5%	0.0412 (0.0107) 18%	0.0227 (0.0035) 79%	0.0207 (0.0016) 86%

¹ Slopes to average triplicate Y values and treat as a single data point in order to test whether the slopes are significantly different

² Data in parentheses is the +/- standard error of slopes

³ Percent inhibition compared to migration rate observed in the presence of EGF alone

ADAM-20 and -23dis-Fc polypeptides showed the greatest inhibition of both EGF- and PMA-induced endothelial migration at 15 µg/ml. ADAM-15 and -17dis-Fc polypeptides were less

effective at inhibiting endothelial cell migration at 15 µg/ml. Hu IgG did not inhibit EGF- or PMA-induced endothelial cell migration in any of the experiments performed where it was included as a control Fc protein.

5

EXAMPLE 4

Activity of ADAM Disintegrin Domain Polypeptides In a Corneal Pocket Assay

A mouse corneal pocket assay is used to quantitate the inhibition of angiogenesis by ADAM disintegrin-Fc polypeptides in vivo. In this assay, agents to be tested for angiogenic or anti-angiogenic activity are immobilized in a slow release form in a hydon pellet, which is implanted into
10 micropockets created in the corneal epithelium of anesthetized mice. Vascularization is measured as the appearance, density, and extent of vessel ingrowth from the vascularized corneal limbus into the normally avascular cornea.

Hydon pellets, as described in Kenyon et al., Invest Ophthalmol. & Visual Science 37:1625, 1996, incorporate sucralfate with bFGF (90 ng/pellet), bFGF and IgG (11 µg/pellet, control), or bFGF
15 and a range of concentrations of ADAM disintegrin-Fc polypeptide. The pellets are surgically implanted into corneal stromal micropockets created by micro-dissection 1 mm medial to the lateral corneal limbus of 6-8 week old male C57BL mice. After five days, at the peak of neovascular response to bFGF, the corneas are photographed, using a Zeiss slit lamp, at an incipient angle of 35-50° from the polar axis in the meridian containing the pellet. Images are digitized and processed by
20 subtractive color filters (Adobe Photoshop 4.0) to delineate established microvessels by hemoglobin content. Image analysis software (Bioquant, Nashville, TN) is used to calculate the fraction of the corneal image that is vascularized, the vessel density within the vascularized area, and the vessel density within the total cornea. The inhibition of bFGF-induced corneal angiogenesis, as a function of the dose of ADAM disintegrin-Fc polypeptide, is determined.

25

EXAMPLE 5

Inhibition of Neovascularization by ADAM Disintegrin Domain Polypeptides in a Murine Transplant Model

Survival of heterotopically transplanted cardiac tissue from one mouse donor to the ear skin of
30 another genetically similar mouse requires adequate neovascularization by the transplanted heart and the surrounding tissue, to promote survival and energy for cardiac muscle function. Inadequate vasculature at the site of transplant causes excessive ischemia to the heart, tissue damage, and failure of the tissue to engraft. Agents that antagonize factors involved in endothelial cell migration and vessel formation can decrease angiogenesis at the site of transplant, thereby limiting graft tissue
35 function and ultimately engraftment itself. A murine heterotopic cardiac isograft model is used to demonstrate the antagonistic effects of ADAM disintegrin-Fc polypeptides on neovascularization. Female BALB/c (≈12 weeks of age) recipients are given neonatal heart grafts from donor mice of the same strain. The donor heart tissue is grafted into the left ear pinnae of the recipient on day 0 and the

mice are divided into two groups. The control group receives human IgG (Hu IgG) while the other group receives ADAM disintegrin-Fc polypeptide, both intraperitoneally. The treatments are continued for five consecutive days. The functionality of the grafts is determined by monitoring visible pulsatile activity on days 7 and 14 post-engraftment. The inhibition of functional engraftment, as a function of the dose of ADAM disintegrin-Fc polypeptide, is determined. The histology of the transplanted hearts is examined in order to visualize the effects of ADAM disintegrin-Fc polypeptides on edema at the site of transplant and host and donor tissue vasculature (using, e.g., Factor VIII staining).

10

EXAMPLE 6**Treatment of Tumors With ADAM Disintegrin Domain Polypeptides**

ADAM disintegrin-Fc polypeptides are tested in animal models of solid tumors. The effect of the ADAM disintegrin-Fc polypeptides is determined by measuring tumor frequency and tumor growth.

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The biological activity of ADAM disintegrin-Fc polypeptides is also demonstrated in other in vitro, ex vivo, and in vivo assays known to the skilled artisan, such as calcium mobilization assays and assays to measure platelet activation, recruitment, or aggregation.

20

The relevant disclosures of publications cited herein are specifically incorporated by reference. The examples presented above are not intended to be exhaustive or to limit the scope of the invention. The skilled artisan will understand that variations and modifications and variations are possible in light of the above teachings, and such modifications and variations are intended to be within the scope of the invention.

25

CLAIMS

We claim:

1. A method of antagonizing the binding of an integrin to its ligands comprising contacting a cell that expresses the integrin with an effective amount of an ADAM disintegrin domain polypeptide.
2. A method of antagonizing the binding of an integrin to its ligands in a mammal in need of such treatment comprising administering an effective amount of an ADAM disintegrin domain polypeptide.
3. The method of claim 2 wherein the mammal is afflicted with a condition selected from the group consisting of ocular disorders, malignant and metastatic conditions, inflammatory diseases, osteoporosis and other conditions mediated by accelerated bone resorption, restenosis, inappropriate platelet activation, recruitment, or aggregation, thrombosis, or a condition requiring tissue repair or wound healing.
4. A method of inhibiting angiogenesis in a mammal in need of such treatment, comprising administering to the mammal an inhibition-effective amount of an ADAM disintegrin domain polypeptide, wherein the disintegrin domain does not contain an RGD sequence.
5. The method of one of claims 1-4 wherein the ADAM disintegrin domain is in the form of a multimer.
6. The method of claim 5 wherein the multimer is a dimer or trimer.
7. The method of claim 5 wherein the multimer comprises an Fc polypeptide or a leucine zipper.
8. The method of one of claims 1-7 wherein the ADAM disintegrin domain is from a human ADAM.
9. The method of claim 8 wherein the ADAM disintegrin domain is from an ADAM selected from the group consisting of ADAM-8, ADAM-9, ADAM-10, ADAM-15, ADAM-17, ADAM-20, ADAM-21, ADAM-22, ADAM-23, and ADAM-29.
10. The method of claim 9 wherein the ADAM disintegrin domain is from ADAM-17, ADAM-20, or ADAM-23.
11. The method of one of claims 1-10 wherein the ADAM disintegrin domain polypeptide comprises an amino acid sequence selected from the group consisting of:
 - (a) amino acids 1-494 of SEQ ID NO:2, amino acids 23-264 of SEQ ID NO:2, amino acids 1-533 of SEQ ID NO:4, amino acids 23-303 of SEQ ID NO:4, amino acids 1-465 of SEQ ID NO:6, amino acids 23-235 of SEQ ID NO:6, amino acids 1-522 of SEQ ID NO:8, amino acids 23-292 of SEQ ID NO:8, amino acids 1-446 of SEQ ID NO:10, amino acids 23-216 of SEQ ID NO:10, amino acids 1-535 of SEQ ID NO:12, amino acids 23-305 of SEQ ID NO:12, amino acids 1-523 of SEQ ID NO:14, amino acids 23-293 of SEQ ID NO:14, amino acids 1-542 of SEQ ID NO:16, amino acids 23-312 of SEQ ID NO:16, amino acids 1-540 of SEQ ID NO:18, amino acids 23-310 of SEQ ID NO:18, amino acids 1-528 of SEQ ID NO:22, amino acids 23-298 of SEQ ID NO:22;

(b) fragments of the polypeptides of (a) wherein said fragments retain at least one ADAMdis activity;

(c) variants of the polypeptides of (a) or (b), wherein said variants retain at least one ADAMdis activity; and

(d) fusion polypeptides comprising the polypeptides of (a), (b), or (c), wherein said fusion polypeptides retain at least one ADAMdis activity.

12. The method of claim 11 wherein the ADAM disintegrin domain comprises an amino acid sequence selected from the group consisting of amino acids 34-91 of SEQ ID NO:2, 34-92 of SEQ ID NO:4, 34-99 of SEQ ID NO:6, 34-92 of SEQ ID NO:8, 34-93 of SEQ ID NO:10, 34-91 of SEQ ID NO:12, 34-91 of SEQ ID NO:14, 34-92 of SEQ ID NO:16, 34-91 of SEQ ID NO:18, or 34-91 of SEQ ID NO:22.

13. The method of one of claims 1-12 wherein the ADAM disintegrin domain polypeptide is a variant that is at least 70%, 80%, 90%, 95%, 98%, or 99% identical in amino acid sequence to a polypeptide selected from the group consisting of:

(a) amino acids 1-494 of SEQ ID NO:2, amino acids 23-264 of SEQ ID NO:2, amino acids 1-533 of SEQ ID NO:4, amino acids 23-303 of SEQ ID NO:4, amino acids 1-465 of SEQ ID NO:6, amino acids 23-235 of SEQ ID NO:6, amino acids 1-522 of SEQ ID NO:8, amino acids 23-292 of SEQ ID NO:8, amino acids 1-446 of SEQ ID NO:10, amino acids 23-216 of SEQ ID NO:10, amino acids 1-535 of SEQ ID NO:12, amino acids 23-305 of SEQ ID NO:12, amino acids 1-523 of SEQ ID NO:14, amino acids 23-293 of SEQ ID NO:14, amino acids 1-542 of SEQ ID NO:16, amino acids 23-312 of SEQ ID NO:16, amino acids 1-540 of SEQ ID NO:18, amino acids 23-310 of SEQ ID NO:18, amino acids 1-528 of SEQ ID NO:22, amino acids 23-298 of SEQ ID NO:22; and

(b) fragments of the polypeptides of (a),
wherein said variant polypeptide retains at least one ADAMdis activity.

14. The method of one of claims 1-10 wherein the ADAM disintegrin domain polypeptide is encoded by a nucleic acid comprising a sequence selected from the group consisting of:

(a) nucleotides 118-1599 of SEQ ID NO:1, nucleotides 184-909 of SEQ ID NO:1, nucleotides 46-1644 of SEQ ID NO:3, nucleotides 112-954 of SEQ ID NO:3, nucleotides 25-1419 of SEQ ID NO:5, nucleotides 91-729 of SEQ ID NO:5, nucleotides 41-1606 of SEQ ID NO:7, nucleotides 107-916 of SEQ ID NO:7, nucleotides 25-1362 of SEQ ID NO:9, nucleotides 91-672 of SEQ ID NO:9, nucleotides 25-1629 of SEQ ID NO:11, nucleotides 91-939 of SEQ ID NO:11, nucleotides 25-1593 of SEQ ID NO:13, nucleotides 91-903 of SEQ ID NO:13, nucleotides 25-1650 of SEQ ID NO:15, nucleotides 91-960 of SEQ ID NO:15, nucleotides 25-1644 of SEQ ID NO:17, nucleotides 91-954 of SEQ ID NO:17, nucleotides 118-1701 of SEQ ID NO:21, nucleotides 184-1011 of SEQ ID NO:21;

(b) sequences which, due to the degeneracy of the genetic code, encode a polypeptide encoded by a nucleic acid of (a); and

(c) sequences that hybridize under conditions of moderate or high stringency to a sequence of (a) or (b) and that encode a polypeptide that retains at least one ADAMdis activity.

15. The method of one of claim 11-14 wherein the ADAMdis activity is selected from the group consisting of integrin binding activity, inhibition of endothelial cell migration, and inhibition of angiogenesis.

16. The method of one of claims 1-15 wherein the ADAM disintegrin domain polypeptide has been produced by culturing a recombinant cell that encodes the ADAM disintegrin domain polypeptide under conditions permitting expression of the ADAM disintegrin domain polypeptide, and recovering the ADAM disintegrin domain polypeptide.

17. The method of one of claims 1-16 wherein the ADAM disintegrin domain polypeptide is present in a composition comprising a pharmaceutically acceptable carrier.

18. The method of claim 2 wherein the mammal has a disease or condition mediated by angiogenesis.

19. The method of claim 18 wherein the disease or condition is characterized by ocular neovascularization.

20. The method of claim 18 wherein the disease or condition is a solid tumor.

21. The method of one of claims 1-20 wherein the method further comprises treating the mammal with radiation.

22. The method of one of claims 1-21 wherein the method further comprises treating the mammal with a second therapeutic agent.

23. The method of claim 22 wherein the second therapeutic agent is selected from the group consisting of alkylating agents, antimetabolites, vinca alkaloids and other plant-derived chemotherapeutics, antitumor antibiotics, antitumor enzymes, topoisomerase inhibitors, platinum analogs, adrenocortical suppressants, hormones and antihormones, antibodies, immunotherapeutics, radiotherapeutics, and biological response modifiers.

24. The method of claim 22 wherein the second therapeutic agent is selected from the group consisting of cisplatin, cyclophosphamide, bleomycin, carboplatin, fluorouracil, 5-fluorouracil, 5-fluorodeoxyuridine, methotrexate, taxol, asparaginase, vincristine, vinblastine, mechlorethamine, melphalan, 5-fluorodeoxyuridine, lymphokines and cytokines such as interleukins, interferons (alpha., beta. or delta.) and TNF, chlorambucil, busulfan, carmustine, lomustine, semustine, streptozocin, dacarbazine, cytarabine, mercaptopurine, thioguanine, vindesine, etoposide, teniposide, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, mitomycin, L-asparaginase, hydroxyurea, methylhydrazine, mitotane, tamoxifen, fluoxymesterone, and COX-2 inhibitors.

25. The method of claim 22 wherein the second therapeutic agent is a polypeptide, including soluble forms thereof, selected from the group consisting of Flt3 ligand, CD40 ligand, interleukin-2, interleukin-12, 4-1BB ligand, anti-4-1BB antibodies, TRAIL, TNF antagonists and TNF receptor antagonists including TNFR/Fc, Tek antagonists, TWEAK antagonists and TWEAK-R antagonists including TWEAK-R/Fc, VEGF antagonists including anti-VEGF antibodies, VEGF receptor antagonists, CD148 binding proteins, and nectin-3 antagonists.

26. The method of claim 2 wherein the ADAM disintegrin domain is administered parenterally.
27. A method for inhibiting the biological activity of an integrin selected from the group consisting of $\alpha_v\beta_3$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, and $\alpha_v\beta_5$ comprising contacting the integrin with an inhibition-effective amount of an ADAM disintegrin domain polypeptide.
28. The method of claim 27 wherein the integrin is $\alpha_v\beta_3$ and wherein the ADAM disintegrin domain does not contain an RGD sequence.
29. The method of claim 28 wherein the ADAM is ADAM-17, ADAM-20, or ADAM-22.
30. The method of claim 27 wherein the integrin is $\alpha_2\beta_1$ and the ADAM is ADAM-23.
31. The method of claim 27 wherein the integrin is $\alpha_5\beta_1$ and the ADAM is ADAM-15, ADAM-21, ADAM-22, or ADAM-23.
32. The method of claim 27 wherein the integrin is $\alpha_6\beta_1$ or $\alpha_6\beta_4$ and the ADAM is ADAM-10, ADAM-17, ADAM-22, or ADAM-23.
33. The method of claim 27 wherein the integrin is $\alpha_v\beta_5$ and the ADAM is ADAM-10, ADAM-15, or ADAM-23.
34. A method for identifying a compound that modulates integrin biological activity comprising:
- (a) combining a test compound with an integrin and an ADAM disintegrin domain polypeptide that binds to the integrin; and
 - (b) determining whether the test compound alters the binding of the ADAM disintegrin domain polypeptide to the integrin.
35. A method for identifying a compound that modulates the interaction between an integrin and an ADAM disintegrin domain comprising:
- (a) combining a test compound with the integrin and an ADAM disintegrin domain polypeptide that binds to the integrin; and
 - (b) determining whether the test compound alters the binding of the ADAM disintegrin domain polypeptide to the integrin.
36. The method of claim 34 or 35 wherein the integrin is present on a cell surface.
37. The method of claim 36 wherein the cell is an endothelial cell.
38. The method of one of claims 34-37 wherein the integrin is selected from the group consisting of $\alpha_v\beta_3$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, and $\alpha_v\beta_5$.
39. The method of one of claims 34-38 wherein the integrin biological activity or integrin binding activity is at least partially inhibited.
40. A method for identifying a compound that inhibits endothelial cell migration and/or angiogenesis comprising:
- (a) combining a test compound with endothelial cells and with an ADAM disintegrin domain polypeptide that binds to endothelial cells; and

(b) determining whether the test compound alters the binding of the ADAM disintegrin domain polypeptide to the endothelial cells.

41. The method of one of claims 34-40 wherein the ADAM disintegrin domain polypeptide comprises an ADAM disintegrin domain from ADAM-8, ADAM-9, ADAM-10, ADAM-15, ADAM-17, ADAM-20, ADAM-21, ADAM-22, ADAM-23, or ADAM-29.

42. The method of claim 41 wherein the ADAM disintegrin domain polypeptide comprises an ADAM disintegrin domain from ADAM-17, ADAM-20, or ADAM-23.

SEQUENCE LISTING

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<120> INTEGRIN ANTAGONISTS

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			40					45					50				
tgt	aag	ctt	aaa	tca	ttt	gct	gag	tgt	gca	tat	ggg	gac	tgt	tgt	aaa	249	
Cys	Lys	Leu	Lys	Ser	Phe	Ala	Glu	Cys	Ala	Tyr	Gly	Asp	Cys	Cys	Lys		
		55				60						65					
gac	tgt	cgg	ttc	ctt	cca	gga	ggg	act	tta	tgc	cga	gga	aaa	acc	agt	297	
Asp	Cys	Arg	Phe	Leu	Pro	Gly	Gly	Thr	Leu	Cys	Arg	Gly	Lys	Thr	Ser		
	70					75					80						
gag	tgt	gat	gtt	cca	gag	tac	tgc	aat	ggg	tct	tct	cag	ttc	tgt	cag	345	
Glu	Cys	Asp	Val	Pro	Glu	Tyr	Cys	Asn	Gly	Ser	Ser	Gln	Phe	Cys	Gln		
	85				90				95						100		
cca	gat	gtt	ttt	att	cag	aat	gga	tat	cct	tgc	cag	aat	aac	aaa	gcc	393	
Pro	Asp	Val	Phe	Ile	Gln	Asn	Gly	Tyr	Pro	Cys	Gln	Asn	Asn	Lys	Ala		
				105				110						115			
tat	tgc	tac	aac	ggc	atg	tgc	cag	tat	tat	gat	gct	caa	tgt	caa	gtc	441	
Tyr	Cys	Tyr	Asn	Gly	Met	Cys	Gln	Tyr	Tyr	Asp	Ala	Gln	Cys	Gln	Val		
			120					125					130				
atc	ttt	ggc	tca	aaa	gcc	aag	gct	gcc	ccc	aaa	gat	tgt	ttc	att	gaa	489	
Ile	Phe	Gly	Ser	Lys	Ala	Lys	Ala	Ala	Pro	Lys	Asp	Cys	Phe	Ile	Glu		
		135				140						145					
gtg	aat	tct	aaa	ggg	gac	aga	ttt	ggc	aat	tgt	ggg	ttc	tct	ggc	aat	537	
Val	Asn	Ser	Lys	Gly	Asp	Arg	Phe	Gly	Asn	Cys	Gly	Phe	Ser	Gly	Asn		
	150					155					160						
gaa	tac	aag	aag	tgt	gcc	act	ggg	aat	gct	ttg	tgt	gga	aag	ctt	cag	585	
Glu	Tyr	Lys	Lys	Cys	Ala	Thr	Gly	Asn	Ala	Leu	Cys	Gly	Lys	Leu	Gln		
	165				170					175					180		
tgt	gag	aat	gta	caa	gag	ata	cct	gta	ttt	gga	att	gtg	cct	gct	att	633	
Cys	Glu	Asn	Val	Gln	Glu	Ile	Pro	Val	Phe	Gly	Ile	Val	Pro	Ala	Ile		
				185					190					195			
att	caa	acg	cct	agt	cga	ggc	acc	aaa	tgt	tgg	ggg	gtg	gat	ttc	cag	681	
Ile	Gln	Thr	Pro	Ser	Arg	Gly	Thr	Lys	Cys	Trp	Gly	Val	Asp	Phe	Gln		
			200					205					210				
cta	gga	tca	gat	gtt	cca	gat	cct	ggg	atg	gtt	aac	gaa	ggc	aca	aaa	729	
Leu	Gly	Ser	Asp	Val	Pro	Asp	Pro	Gly	Met	Val	Asn	Glu	Gly	Thr	Lys		
			215				220					225					
tgt	ggg	gct	gga	aag	atc	tgt	aga	aac	ttc	cag	tgt	gta	gat	gct	tct	777	
Cys	Gly	Ala	Gly	Lys	Ile	Cys	Arg	Asn	Phe	Gln	Cys	Val	Asp	Ala	Ser		
	230					235					240						
gtt	ctg	aat	tat	gac	tgt	gat	gtt	cag	aaa	aag	tgt	cat	gga	cat	ggg	825	
Val	Leu	Asn	Tyr	Asp	Cys	Asp	Val	Gln	Lys	Lys	Cys	His	Gly	His	Gly		
	245				250					255					260		
gta	tgt	aat	agc	aat	aag	aat	tgt	cac	tgt	gaa	aat	ggc	tgg	gct	ccc	873	
Val	Cys	Asn	Ser	Asn	Lys	Asn	Cys	His	Cys	Glu	Asn	Gly	Trp	Ala	Pro		
				265				270						275			
cca	aat	tgt	gag	act	aaa	gga	tac	gga	gga	agt	gtg	gac	agt	gga	cct	921	
Pro	Asn	Cys	Glu	Thr	Lys	Gly	Tyr	Gly	Gly	Ser	Val	Asp	Ser	Gly	Pro		
			280					285					290				

aca tac aat gaa atg aat act gca ttg agg gac gga tct tgt gac aaa 969
 Thr Tyr Asn Glu Met Asn Thr Ala Leu Arg Asp Gly Ser Cys Asp Lys
 295 300 305
 act cac aca tgc cca ccg tgc cca gca cct gaa gcc gag ggc gcg ccg 1017
 Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro
 310 315 320
 tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc 1065
 Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
 325 330 335 340
 cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac 1113
 Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
 345 350 355
 cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat 1161
 Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
 360 365 370
 gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg 1209
 Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
 375 380 385
 gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag 1257
 Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 390 395 400
 tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa 1305
 Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
 405 410 415 420
 acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc 1353
 Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 425 430 435
 ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc 1401
 Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 440 445 450
 tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag 1449
 Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 455 460 465
 agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg 1497
 Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 470 475 480
 gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag 1545
 Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 485 490 495 500
 agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag 1593
 Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 505 510 515
 gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt 1641
 Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 520 525 530
 aaa tga actagagcgg ccgctacaga t 1668
 Lys

<210> 4

<211> 533
 <212> PRT
 <213> Artificial Sequence
 <223> Description of Artificial Sequence: fusion
 polypeptide

<400> 4

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Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
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Gly Ser Thr Gly Thr Ser Cys Gly Asn Lys Leu Val Asp Ala Gly Glu
      20      25      30
Glu Cys Asp Cys Gly Thr Pro Lys Glu Cys Glu Leu Asp Pro Cys Cys
      35      40      45
Glu Gly Ser Thr Cys Lys Leu Lys Ser Phe Ala Glu Cys Ala Tyr Gly
      50      55      60
Asp Cys Cys Lys Asp Cys Arg Phe Leu Pro Gly Gly Thr Leu Cys Arg
      65      70      75      80
Gly Lys Thr Ser Glu Cys Asp Val Pro Glu Tyr Cys Asn Gly Ser Ser
      85      90      95
Gln Phe Cys Gln Pro Asp Val Phe Ile Gln Asn Gly Tyr Pro Cys Gln
      100      105      110
Asn Asn Lys Ala Tyr Cys Tyr Asn Gly Met Cys Gln Tyr Tyr Asp Ala
      115      120      125
Gln Cys Gln Val Ile Phe Gly Ser Lys Ala Lys Ala Ala Pro Lys Asp
      130      135      140
Cys Phe Ile Glu Val Asn Ser Lys Gly Asp Arg Phe Gly Asn Cys Gly
      145      150      155      160
Phe Ser Gly Asn Glu Tyr Lys Lys Cys Ala Thr Gly Asn Ala Leu Cys
      165      170      175
Gly Lys Leu Gln Cys Glu Asn Val Gln Glu Ile Pro Val Phe Gly Ile
      180      185      190
Val Pro Ala Ile Ile Gln Thr Pro Ser Arg Gly Thr Lys Cys Trp Gly
      195      200      205
Val Asp Phe Gln Leu Gly Ser Asp Val Pro Asp Pro Gly Met Val Asn
      210      215      220
Glu Gly Thr Lys Cys Gly Ala Gly Lys Ile Cys Arg Asn Phe Gln Cys
      225      230      235      240
Val Asp Ala Ser Val Leu Asn Tyr Asp Cys Asp Val Gln Lys Lys Cys
      245      250      255
His Gly His Gly Val Cys Asn Ser Asn Lys Asn Cys His Cys Glu Asn
      260      265      270
Gly Trp Ala Pro Pro Asn Cys Glu Thr Lys Gly Tyr Gly Gly Ser Val
      275      280      285
Asp Ser Gly Pro Thr Tyr Asn Glu Met Asn Thr Ala Leu Arg Asp Gly
      290      295      300
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala
      305      310      315      320
Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
      325      330      335
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
      340      345      350
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
      355      360      365
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
      370      375      380
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
      385      390      395      400
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
      405      410      415
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
      420      425      430
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
      435      440      445
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
      450      455      460

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Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
 465 470 475 480
 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
 485 490 495
 Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
 500 505 510
 Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
 515 520 525
 Leu Ser Pro Gly Lys
 530

<210> 5

<211> 1443

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: fusion polypeptide

<220>

<221> CDS

<222> (25)..(1422)

<400> 5

gtcgacccaa gctggctagc cacc atg gag aca gac aca ctc ctg cta tgg 51
 Met Glu Thr Asp Thr Leu Leu Leu Trp
 1 5

gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act agt tgt gga aat 99
 Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Thr Ser Cys Gly Asn
 10 15 20 25

gga atg gta gaa caa ggt gaa gaa tgt gat tgt ggc tat agt gac cag 147
 Gly Met Val Glu Gln Gly Glu Glu Cys Asp Cys Gly Tyr Ser Asp Gln
 30 35 40

tgt aaa gat gaa tgc tgc ttc gat gca aat caa cca gag gga aga aaa 195
 Cys Lys Asp Glu Cys Cys Phe Asp Ala Asn Gln Pro Glu Gly Arg Lys
 45 50 55

tgc aaa ctg aaa cct ggg aaa cag tgc agt cca agt caa ggt cct tgt 243
 Cys Lys Leu Lys Pro Gly Lys Gln Cys Ser Pro Ser Gln Gly Pro Cys
 60 65 70

tgt aca gca cag tgt gca ttc aag tca aag tct gag aag tgt cgg gat 291
 Cys Thr Ala Gln Cys Ala Phe Lys Ser Lys Ser Glu Lys Cys Arg Asp
 75 80 85

gat tca gac tgt gca agg gaa gga ata tgt aat ggc ttc aca gct ctc 339
 Asp Ser Asp Cys Ala Arg Glu Gly Ile Cys Asn Gly Phe Thr Ala Leu
 90 95 100 105

tgc cca gca tct gac cct aaa cca aac ttc aca gac tgt aat agg cat 387
 Cys Pro Ala Ser Asp Pro Lys Pro Asn Phe Thr Asp Cys Asn Arg His
 110 115 120

aca caa gtg tgc att aat ggg caa tgt gca ggt tct atc tgt gag aaa 435
 Thr Gln Val Cys Ile Asn Gly Gln Cys Ala Gly Ser Ile Cys Glu Lys
 125 130 135

tat ggc tta gag gag tgt acg tgt gcc agt tct gat ggc aaa gat gat 483
 Tyr Gly Leu Glu Glu Cys Thr Cys Ala Ser Ser Asp Gly Lys Asp Asp
 140 145 150

aaa gaa tta tgc cat gta tgc tgt atg aag aaa atg gac cca tca act	531
Lys Glu Leu Cys His Val Cys Cys Met Lys Lys Met Asp Pro Ser Thr	
155 160 165	
tgt gcc agt aca ggg tct gtg cag tgg agt agg cac ttc agt ggt cga	579
Cys Ala Ser Thr Gly Ser Val Gln Trp Ser Arg His Phe Ser Gly Arg	
170 175 180 185	
acc atc acc ctg caa cct gga tcc cct tgc aac gat ttt aga ggt tac	627
Thr Ile Thr Leu Gln Pro Gly Ser Pro Cys Asn Asp Phe Arg Gly Tyr	
190 195 200	
tgt gat gtt ttc atg cgg tgc aga tta gta gat gct gat ggt cct cta	675
Cys Asp Val Phe Met Arg Cys Arg Leu Val Asp Ala Asp Gly Pro Leu	
205 210 215	
gct agg ctt aaa aaa gca att ttt agt cca gag ctc tat gaa aac att	723
Ala Arg Leu Lys Lys Ala Ile Phe Ser Pro Glu Leu Tyr Glu Asn Ile	
220 225 230	
gct gaa aga tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca	771
Ala Glu Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala	
235 240 245	
cct gaa gcc gag ggc gcg ccg tca gtc ttc ctc ttc ccc cca aaa ccc	819
Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro	
250 255 260 265	
aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg	867
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val	
270 275 280	
gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg	915
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val	
285 290 295	
gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag	963
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln	
300 305 310	
tac aac agc acg tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag	1011
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln	
315 320 325	
gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc	1059
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala	
330 335 340 345	
ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc	1107
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro	
350 355 360	
cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc	1155
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr	
365 370 375	
aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc	1203
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser	
380 385 390	
gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac	1251
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr	
395 400 405	
aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac	1299

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
 410 415 420 425
 agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc 1347
 Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
 430 435 440
 tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag 1395
 Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
 445 450 455
 agc ctc tcc ctg tct ccg ggt aaa tga actagagcgg ccgctacaga t 1443
 Ser Leu Ser Leu Ser Pro Gly Lys
 460 465

<210> 6

<211> 465

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: fusion polypeptide

<400> 6

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
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 Gly Ser Thr Gly Thr Ser Cys Gly Asn Gly Met Val Glu Gln Gly Glu
 20 25 30
 Glu Cys Asp Cys Gly Tyr Ser Asp Gln Cys Lys Asp Glu Cys Cys Phe
 35 40 45
 Asp Ala Asn Gln Pro Glu Gly Arg Lys Cys Lys Leu Lys Pro Gly Lys
 50 55 60
 Gln Cys Ser Pro Ser Gln Gly Pro Cys Cys Thr Ala Gln Cys Ala Phe
 65 70 75 80
 Lys Ser Lys Ser Glu Lys Cys Arg Asp Asp Ser Asp Cys Ala Arg Glu
 85 90 95
 Gly Ile Cys Asn Gly Phe Thr Ala Leu Cys Pro Ala Ser Asp Pro Lys
 100 105 110
 Pro Asn Phe Thr Asp Cys Asn Arg His Thr Gln Val Cys Ile Asn Gly
 115 120 125
 Gln Cys Ala Gly Ser Ile Cys Glu Lys Tyr Gly Leu Glu Glu Cys Thr
 130 135 140
 Cys Ala Ser Ser Asp Gly Lys Asp Asp Lys Glu Leu Cys His Val Cys
 145 150 155 160
 Cys Met Lys Lys Met Asp Pro Ser Thr Cys Ala Ser Thr Gly Ser Val
 165 170 175
 Gln Trp Ser Arg His Phe Ser Gly Arg Thr Ile Thr Leu Gln Pro Gly
 180 185 190
 Ser Pro Cys Asn Asp Phe Arg Gly Tyr Cys Asp Val Phe Met Arg Cys
 195 200 205
 Arg Leu Val Asp Ala Asp Gly Pro Leu Ala Arg Leu Lys Lys Ala Ile
 210 215 220
 Phe Ser Pro Glu Leu Tyr Glu Asn Ile Ala Glu Arg Ser Cys Asp Lys
 225 230 235 240
 Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro
 245 250 255
 Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
 260 265 270
 Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
 275 280 285
 Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
 290 295 300
 Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
 305 310 315 320
 Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 325 330 335

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
 340 345 350
 Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 355 360 365
 Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 370 375 380
 Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 385 390 395 400
 Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 405 410 415
 Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 420 425 430
 Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 435 440 445
 Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 450 455 460
 Lys
 465

<210> 7
 <211> 1638
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: fusion
 polypeptide

<220>
 <221> CDS
 <222> (41)..(1609)

<400> 7
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 ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act 103
 Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Thr 20
 10 15

 agt tgc gga aat atg ttt gtg gag ccg ggc gag cag tgt gac tgt ggc 151
 Ser Cys Gly Asn Met Phe Val Glu Pro Gly Glu Gln Cys Asp Cys Gly 35
 25 30

 ttc ctg gat gac tgc gtc gat ccc tgc tgt gat tct ttg acc tgc cag 199
 Phe Leu Asp Asp Cys Val Asp Pro Cys Cys Asp Ser Leu Thr Cys Gln 50
 40 45

 ctg agg cca ggt gca cag tgt gca tct gac gga ccc tgt tgt caa aat 247
 Leu Arg Pro Gly Ala Gln Cys Ala Ser Asp Gly Pro Cys Cys Gln Asn 65
 55 60 65

 tgc cag ctg cgc ccg tct ggc tgg cag tgt cgt cct acc aga ggg gat 295
 Cys Gln Leu Arg Pro Ser Gly Trp Gln Cys Arg Pro Thr Arg Gly Asp 85
 70 75 80

 tgt gac ttg cct gaa ttc tgc cca gga gac agc tcc cag tgt ccc cct 343
 Cys Asp Leu Pro Glu Phe Cys Pro Gly Asp Ser Ser Gln Cys Pro Pro 100
 90 95

 gat gtc agc cta ggg gat ggc gag ccc tgc gct ggc ggg caa gct gtg 391
 Asp Val Ser Leu Gly Asp Gly Glu Pro Cys Ala Gly Gly Gln Ala Val 115
 105 110 115

tgc atg cac ggg cgt tgt gcc tcc tat gcc cag cag tgc cag tca ctt	439
Cys Met His Gly Arg Cys Ala Ser Tyr Ala Gln Gln Cys Gln Ser Leu	
120 125 130	
tgg gga cct gga gcc cag ccc gct gcg cca ctt tgc ctc cag aca gct	487
Trp Gly Pro Gly Ala Gln Pro Ala Ala Pro Leu Cys Leu Gln Thr Ala	
135 140 145	
aat act cgg gga aat gct ttt ggg agc tgt ggg cgc aac ccc agt ggc	535
Asn Thr Arg Gly Asn Ala Phe Gly Ser Cys Gly Arg Asn Pro Ser Gly	
150 155 160 165	
agt tat gtg tcc tgc acc cct aga gat gcc att tgt ggg cag ctc cag	583
Ser Tyr Val Ser Cys Thr Pro Arg Asp Ala Ile Cys Gly Gln Leu Gln	
170 175 180	
tgc cag aca ggt agg acc cag cct ctg ctg ggc tcc atc cgg gat cta	631
Cys Gln Thr Gly Arg Thr Gln Pro Leu Leu Gly Ser Ile Arg Asp Leu	
185 190 195	
ctc tgg gag aca ata gat gtg aat ggg act gag ctg aac tgc agc tgg	679
Leu Trp Glu Thr Ile Asp Val Asn Gly Thr Glu Leu Asn Cys Ser Trp	
200 205 210	
gtg cac ctg gac ctg ggc agt gat gtg gcc cag ccc ctc ctg act ctg	727
Val His Leu Asp Leu Gly Ser Asp Val Ala Gln Pro Leu Leu Thr Leu	
215 220 225	
cct ggc aca gcc tgt ggc cct ggc ctg gtg tgt ata gac cat cga tgc	775
Pro Gly Thr Ala Cys Gly Pro Gly Leu Val Cys Ile Asp His Arg Cys	
230 235 240 245	
cag cgt gtg gat ctc ctg ggg gca cag gaa tgt cga agc aaa tgc cat	823
Gln Arg Val Asp Leu Leu Gly Ala Gln Glu Cys Arg Ser Lys Cys His	
250 255 260	
gga cat ggg gtc tgt gac agc aac agg cac tgc tac tgt gag gag ggc	871
Gly His Gly Val Cys Asp Ser Asn Arg His Cys Tyr Cys Glu Glu Gly	
265 270 275	
tgg gca ccc cct gac tgc acc act cag ctc aaa gca acc agc tcc aga	919
Trp Ala Pro Pro Asp Cys Thr Thr Gln Leu Lys Ala Thr Ser Ser Arg	
280 285 290	
tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa gcc	967
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala	
295 300 305	
gag ggc gcg ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc	1015
Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr	
310 315 320 325	
ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg	1063
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val	
330 335 340	
agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg	1111
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val	
345 350 355	
gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc	1159
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser	
360 365 370	
acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg	1207

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Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
 375                               380                               385

aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc 1255
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
390                               395                               400                               405

ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca 1303
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
                               410                               415                               420

cag gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag 1351
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
                               425                               430                               435

gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc 1399
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
                               440                               445                               450

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg 1447
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                               455                               460                               465

cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tat agc aag ctc 1495
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
470                               475                               480                               485

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc 1543
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
                               490                               495                               500

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc 1591
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
                               505                               510                               515

ctg tct ccg ggt aaa tga actagagcgg ccgccaccgc ggtggagct 1638
Leu Ser Pro Gly Lys
                               520

```

<210> 8

<211> 522

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: fusion
polypeptide

<400> 8

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Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1                               5                               10                               15
Gly Ser Thr Gly Thr Ser Cys Gly Asn Met Phe Val Glu Pro Gly Glu
                               20                               25                               30
Gln Cys Asp Cys Gly Phe Leu Asp Asp Cys Val Asp Pro Cys Cys Asp
                               35                               40                               45
Ser Leu Thr Cys Gln Leu Arg Pro Gly Ala Gln Cys Ala Ser Asp Gly
                               50                               55                               60
Pro Cys Cys Gln Asn Cys Gln Leu Arg Pro Ser Gly Trp Gln Cys Arg
                               65                               70                               75                               80
Pro Thr Arg Gly Asp Cys Asp Leu Pro Glu Phe Cys Pro Gly Asp Ser
                               85                               90                               95
Ser Gln Cys Pro Pro Asp Val Ser Leu Gly Asp Gly Glu Pro Cys Ala
                               100                               105                               110
Gly Gly Gln Ala Val Cys Met His Gly Arg Cys Ala Ser Tyr Ala Gln
                               115                               120                               125
Gln Cys Gln Ser Leu Trp Gly Pro Gly Ala Gln Pro Ala Ala Pro Leu
                               130                               135                               140

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Cys Leu Gln Thr Ala Asn Thr Arg Gly Asn Ala Phe Gly Ser Cys Gly
 145 150 155 160
 Arg Asn Pro Ser Gly Ser Tyr Val Ser Cys Thr Pro Arg Asp Ala Ile
 165 170 175
 Cys Gly Gln Leu Gln Cys Gln Thr Gly Arg Thr Gln Pro Leu Leu Gly
 180 185 190
 Ser Ile Arg Asp Leu Leu Trp Glu Thr Ile Asp Val Asn Gly Thr Glu
 195 200 205
 Leu Asn Cys Ser Trp Val His Leu Asp Leu Gly Ser Asp Val Ala Gln
 210 215 220
 Pro Leu Leu Thr Leu Pro Gly Thr Ala Cys Gly Pro Gly Leu Val Cys
 225 230 235 240
 Ile Asp His Arg Cys Gln Arg Val Asp Leu Leu Gly Ala Gln Glu Cys
 245 250 255
 Arg Ser Lys Cys His Gly His Gly Val Cys Asp Ser Asn Arg His Cys
 260 265 270
 Tyr Cys Glu Glu Gly Trp Ala Pro Pro Asp Cys Thr Thr Gln Leu Lys
 275 280 285
 Ala Thr Ser Ser Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
 290 295 300
 Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro
 305 310 315 320
 Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
 325 330 335
 Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
 340 345 350
 Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
 355 360 365
 Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
 370 375 380
 His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
 385 390 395 400
 Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
 405 410 415
 Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
 420 425 430
 Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 435 440 445
 Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 450 455 460
 Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
 465 470 475 480
 Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 485 490 495
 Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 500 505 510
 Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 515 520

<210> 9

<211> 1386

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: fusion polypeptide

<220>

<221> CDS

<222> (25)..(1365)

<400> 9

gtcgacccaa gctggctagc cacc atg gag aca gac aca ctc ctg cta tgg 51

	Met	Glu	Thr	Asp	Thr	Leu	Leu	Leu	Trp	
	1				5					
gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act agt tgt ggg aac	99									
Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Thr Ser Cys Gly Asn										
10 15 20 25										
tcg agg gtg gat gaa gga gaa gag tgt gat cct ggc atc atg tat ctg	147									
Ser Arg Val Asp Glu Gly Glu Glu Cys Asp Pro Gly Ile Met Tyr Leu										
30 35 40										
aac aac gac acc tgc tgc aac agc gac tgc acg ttg aag gaa ggt gtc	195									
Asn Asn Asp Thr Cys Cys Asn Ser Asp Cys Thr Leu Lys Glu Gly Val										
45 50 55										
cag tgc agt gac agg aac agt cct tgc tgt aaa aac tgt cag ttt gag	243									
Gln Cys Ser Asp Arg Asn Ser Pro Cys Cys Lys Asn Cys Gln Phe Glu										
60 65 70										
act gcc cag aag aag tgc cag gag gcg att aat gct act tgc aaa ggc	291									
Thr Ala Gln Lys Lys Cys Gln Glu Ala Ile Asn Ala Thr Cys Lys Gly										
75 80 85										
gtg tcc tac tgc aca ggt aat agc agt gag tgc ccg cct cca gga aat	339									
Val Ser Tyr Cys Thr Gly Asn Ser Ser Glu Cys Pro Pro Pro Gly Asn										
90 95 100 105										
gct gaa gat gac act gtt tgc ttg gat ctt ggc aag tgt aag gat ggg	387									
Ala Glu Asp Asp Thr Val Cys Leu Asp Leu Gly Lys Cys Lys Asp Gly										
110 115 120										
aaa tgc atc cct ttc tgc gag agg gaa cag cag ctg gag tcc tgt gca	435									
Lys Cys Ile Pro Phe Cys Glu Arg Glu Gln Gln Leu Glu Ser Cys Ala										
125 130 135										
tgt aat gaa act gac aac tcc tgc aag gtg tgc tgc agg gac ctt tcc	483									
Cys Asn Glu Thr Asp Asn Ser Cys Lys Val Cys Cys Arg Asp Leu Ser										
140 145 150										
ggc cgc tgt gtg ccc tat gtc gat gct gaa caa aag aac tta ttt ttg	531									
Gly Arg Cys Val Pro Tyr Val Asp Ala Glu Gln Lys Asn Leu Phe Leu										
155 160 165										
agg aaa gga aag ccc tgt aca gta gga ttt tgt gac atg aat ggc aaa	579									
Arg Lys Gly Lys Pro Cys Thr Val Gly Phe Cys Asp Met Asn Gly Lys										
170 175 180 185										
tgt gag aaa cga gta cag gat gta att gaa cga ttt tgg gat ttc att	627									
Cys Glu Lys Arg Val Gln Asp Val Ile Glu Arg Phe Trp Asp Phe Ile										
190 195 200										
gac cag ctg agc atc aat act ttt gga aag ttt tta gca gac aac aga	675									
Asp Gln Leu Ser Ile Asn Thr Phe Gly Lys Phe Leu Ala Asp Asn Arg										
205 210 215										
tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa gcc	723									
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala										
220 225 230										
gag ggc gcg ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc	771									
Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr										
235 240 245										
ctc atg atc tcc ccg acc cct gag gtc aca tgc gtg gtg gtg gac gtg	819									
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val										
250 255 260 265										

agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg 867
 Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
 270 275 280

gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc 915
 Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
 285 290 295

acg tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg 963
 Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
 300 305 310

aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc 1011
 Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
 315 320 325

ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca 1059
 Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
 330 335 340 345

cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag 1107
 Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
 350 355 360

gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc 1155
 Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
 365 370 375

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg 1203
 Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
 380 385 390

cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc 1251
 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
 395 400 405

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc 1299
 Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
 410 415 420 425

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc 1347
 Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
 430 435 440

ctg tct ccg ggt aaa tga actagagcgg ccgctacaga t 1386
 Leu Ser Pro Gly Lys
 445

<210> 10

<211> 446

<212> PRT

<213> Artificial Sequence

 <223> Description of Artificial Sequence: fusion
 polypeptide

<400> 10

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1 5 10 15
 Gly Ser Thr Gly Thr Ser Cys Gly Asn Ser Arg Val Asp Glu Gly Glu
 20 25 30
 Glu Cys Asp Pro Gly Ile Met Tyr Leu Asn Asn Asp Thr Cys Cys Asn
 35 40 45
 Ser Asp Cys Thr Leu Lys Glu Gly Val Gln Cys Ser Asp Arg Asn Ser
 50 55 60

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Pro Cys Cys Lys Asn Cys Gln Phe Glu Thr Ala Gln Lys Lys Cys Gln
65          70          75          80
Glu Ala Ile Asn Ala Thr Cys Lys Gly Val Ser Tyr Cys Thr Gly Asn
85          90          95
Ser Ser Glu Cys Pro Pro Pro Gly Asn Ala Glu Asp Asp Thr Val Cys
100        105        110
Leu Asp Leu Gly Lys Cys Lys Asp Gly Lys Cys Ile Pro Phe Cys Glu
115        120        125
Arg Glu Gln Gln Leu Glu Ser Cys Ala Cys Asn Glu Thr Asp Asn Ser
130        135        140
Cys Lys Val Cys Cys Arg Asp Leu Ser Gly Arg Cys Val Pro Tyr Val
145        150        155        160
Asp Ala Glu Gln Lys Asn Leu Phe Leu Arg Lys Gly Lys Pro Cys Thr
165        170        175
Val Gly Phe Cys Asp Met Asn Gly Lys Cys Glu Lys Arg Val Gln Asp
180        185        190
Val Ile Glu Arg Phe Trp Asp Phe Ile Asp Gln Leu Ser Ile Asn Thr
195        200        205
Phe Gly Lys Phe Leu Ala Asp Asn Arg Ser Cys Asp Lys Thr His Thr
210        215        220
Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe
225        230        235        240
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
245        250        255
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
260        265        270
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
275        280        285
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
290        295        300
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305        310        315        320
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
325        330        335
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
340        345        350
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
355        360        365
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
370        375        380
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
385        390        395        400
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
405        410        415
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
420        425        430
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435        440        445

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<210> 11

<211> 1653

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: fusion
polypeptide

<220>

<221> CDS

<222> (25)..(1632)

<400> 11

gtcgacccaa gctggctagc cacc atg gag aca gac aca ctc ctg cta tgg 51

Met Glu Thr Asp Thr Leu Leu Leu Trp																		
1										5								
gta	ctg	ctg	ctc	tgg	gtt	cca	ggt	tcc	act	ggt	act	agt	tgt	ggg	aat	99		
Val	Leu	Leu	Leu	Trp	Val	Pro	Gly	Ser	Thr	Gly	Thr	Ser	Cys	Gly	Asn			
10					15					20					25			
cta	gtg	gtt	gaa	gaa	ggg	gag	gaa	tgt	gac	tgt	gga	acc	ata	cgg	cag	147		
Leu	Val	Val	Glu	Gly	Glu	Glu	Cys	Asp	Cys	Gly	Thr	Ile	Arg	Gln				
			30					35						40				
tgt	gca	aaa	gat	ccc	tgt	tgt	ctg	tta	aac	tgt	act	cta	cat	cct	ggg	195		
Cys	Ala	Lys	Asp	Pro	Cys	Cys	Leu	Leu	Asn	Cys	Thr	Leu	His	Pro	Gly			
			45				50						55					
gct	gct	tgt	gct	ttt	gga	ata	tgt	tgc	aaa	gac	tgc	aaa	ttt	ctg	cca	243		
Ala	Ala	Cys	Ala	Phe	Gly	Ile	Cys	Cys	Lys	Asp	Cys	Lys	Phe	Leu	Pro			
			60				65						70					
tca	gga	act	tta	tgt	aga	caa	caa	gtt	ggt	gaa	tgt	gac	ctt	cca	gag	291		
Ser	Gly	Thr	Leu	Cys	Arg	Gln	Gln	Val	Gly	Glu	Cys	Asp	Leu	Pro	Glu			
	75					80					85							
tgg	tgc	aat	ggg	aca	tcc	cat	caa	tgc	cca	gat	gat	gtg	tat	gtg	cag	339		
Trp	Cys	Asn	Gly	Thr	Ser	His	Gln	Cys	Pro	Asp	Asp	Val	Tyr	Val	Gln			
	90				95					100					105			
gac	ggg	atc	tcc	tgt	aat	gtg	aat	gcc	ttc	tgc	tat	gaa	aag	acg	tgt	387		
Asp	Gly	Ile	Ser	Cys	Asn	Val	Asn	Ala	Phe	Cys	Tyr	Glu	Lys	Thr	Cys			
				110					115					120				
aat	aac	cat	gat	ata	caa	tgt	aaa	gag	att	ttt	ggc	caa	gat	gca	agg	435		
Asn	Asn	His	Asp	Ile	Gln	Cys	Lys	Glu	Ile	Phe	Gly	Gln	Asp	Ala	Arg			
			125					130					135					
agt	gca	tct	cag	agt	tgc	tac	caa	gaa	atc	aac	acc	caa	gga	aac	cgt	483		
Ser	Ala	Ser	Gln	Ser	Cys	Tyr	Gln	Glu	Ile	Asn	Thr	Gln	Gly	Asn	Arg			
		140					145					150						
ttc	ggt	cac	tgt	ggt	att	gta	ggc	aca	aca	tat	gta	aaa	tgt	tgg	acc	531		
Phe	Gly	His	Cys	Gly	Ile	Val	Gly	Thr	Thr	Tyr	Val	Lys	Cys	Trp	Thr			
	155					160					165							
cct	gat	atc	atg	tgt	ggg	agg	gtt	cag	tgt	gaa	aat	gtg	gga	gta	att	579		
Pro	Asp	Ile	Met	Cys	Gly	Arg	Val	Gln	Cys	Glu	Asn	Val	Gly	Val	Ile			
	170				175					180					185			
ccc	aat	ctg	ata	gag	cat	tct	aca	gtg	cag	cag	ttt	cac	ctc	aat	gac	627		
Pro	Asn	Leu	Ile	Glu	His	Ser	Thr	Val	Gln	Gln	Phe	His	Leu	Asn	Asp			
				190					195					200				
acc	act	tgc	tgg	ggc	act	gat	tat	cat	tta	ggg	atg	gct	ata	cct	gat	675		
Thr	Thr	Cys	Trp	Gly	Thr	Asp	Tyr	His	Leu	Gly	Met	Ala	Ile	Pro	Asp			
			205					210					215					
att	ggt	gag	gtg	aaa	gat	ggc	aca	gta	tgt	ggt	cca	gaa	aag	atc	tgc	723		
Ile	Gly	Glu	Val	Lys	Asp	Gly	Thr	Val	Cys	Gly	Pro	Glu	Lys	Ile	Cys			
		220					225					230						
atc	cgt	aag	aag	tgt	gcc	agt	atg	gtt	cat	ctg	tca	caa	gcc	tgt	cag	771		
Ile	Arg	Lys	Lys	Cys	Ala	Ser	Met	Val	His	Leu	Ser	Gln	Ala	Cys	Gln			
	235					240					245							
cct	aag	acc	tgc	aac	atg	agg	gga	atc	tgc	aac	aac	aaa	caa	cac	tgt	819		
Pro	Lys	Thr	Cys	Asn	Met	Arg	Gly	Ile	Cys	Asn	Asn	Lys	Gln	His	Cys			
	250				255					260					265			

cac tgc aac cat gaa tgg gca ccc cca tac tgc aag gac aaa ggc tat	867
His Cys Asn His Glu Trp Ala Pro Pro Tyr Cys Lys Asp Lys Gly Tyr	
270 275 280	
gga ggt agt gct gat agt ggc cca cct cct aag aac aac atg gaa gga	915
Gly Gly Ser Ala Asp Ser Gly Pro Pro Pro Lys Asn Asn Met Glu Gly	
285 290 295	
tta aat gtg atg gga aag ttg cgt gga tct tgt gac aaa act cac aca	963
Leu Asn Val Met Gly Lys Leu Arg Gly Ser Cys Asp Lys Thr His Thr	
300 305 310	
tgc cca ccg tgc cca gca cct gaa gcc gag ggc gcg ccg tca gtc ttc	1011
Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe	
315 320 325	
ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct	1059
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro	
330 335 340 345	
gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc	1107
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val	
350 355 360	
aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca	1155
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr	
365 370 375	
aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg gtc agc gtc	1203
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val	
380 385 390	
ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc	1251
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys	
395 400 405	
aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc	1299
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser	
410 415 420 425	
aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca	1347
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro	
430 435 440	
tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc	1395
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val	
445 450 455	
aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg	1443
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly	
460 465 470	
cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac	1491
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp	
475 480 485	
ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg	1539
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp	
490 495 500 505	
cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac	1587
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His	
510 515 520	
aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga	1632

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 525 530 535

actagagcgg ccgctacaga t

1653

<210> 12
 <211> 535
 <212> PRT
 <213> Artificial Sequence
 <223> Description of Artificial Sequence: fusion
 polypeptide

<400> 12
 Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1 5 10 15
 Gly Ser Thr Gly Thr Ser Cys Gly Asn Leu Val Val Glu Glu Gly Glu
 20 25 30
 Glu Cys Asp Cys Gly Thr Ile Arg Gln Cys Ala Lys Asp Pro Cys Cys
 35 40 45
 Leu Leu Asn Cys Thr Leu His Pro Gly Ala Ala Cys Ala Phe Gly Ile
 50 55 60
 Cys Cys Lys Asp Cys Lys Phe Leu Pro Ser Gly Thr Leu Cys Arg Gln
 65 70 75 80
 Gln Val Gly Glu Cys Asp Leu Pro Glu Trp Cys Asn Gly Thr Ser His
 85 90 95
 Gln Cys Pro Asp Asp Val Tyr Val Gln Asp Gly Ile Ser Cys Asn Val
 100 105 110
 Asn Ala Phe Cys Tyr Glu Lys Thr Cys Asn Asn His Asp Ile Gln Cys
 115 120 125
 Lys Glu Ile Phe Gly Gln Asp Ala Arg Ser Ala Ser Gln Ser Cys Tyr
 130 135 140
 Gln Glu Ile Asn Thr Gln Gly Asn Arg Phe Gly His Cys Gly Ile Val
 145 150 155 160
 Gly Thr Thr Tyr Val Lys Cys Trp Thr Pro Asp Ile Met Cys Gly Arg
 165 170 175
 Val Gln Cys Glu Asn Val Gly Val Ile Pro Asn Leu Ile Glu His Ser
 180 185 190
 Thr Val Gln Gln Phe His Leu Asn Asp Thr Thr Cys Trp Gly Thr Asp
 195 200 205
 Tyr His Leu Gly Met Ala Ile Pro Asp Ile Gly Glu Val Lys Asp Gly
 210 215 220
 Thr Val Cys Gly Pro Glu Lys Ile Cys Ile Arg Lys Lys Cys Ala Ser
 225 230 235 240
 Met Val His Leu Ser Gln Ala Cys Gln Pro Lys Thr Cys Asn Met Arg
 245 250 255
 Gly Ile Cys Asn Asn Lys Gln His Cys His Cys Asn His Glu Trp Ala
 260 265 270
 Pro Pro Tyr Cys Lys Asp Lys Gly Tyr Gly Gly Ser Ala Asp Ser Gly
 275 280 285
 Pro Pro Pro Lys Asn Asn Met Glu Gly Leu Asn Val Met Gly Lys Leu
 290 295 300
 Arg Gly Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
 305 310 315 320
 Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
 325 330 335
 Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
 340 345 350
 Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
 355 360 365
 Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
 370 375 380
 Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
 385 390 395 400
 Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
 405 410 415

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
 420 425 430
 Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
 435 440 445
 Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
 450 455 460
 Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
 465 470 475 480
 Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
 485 490 495
 Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
 500 505 510
 Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
 515 520 525
 Leu Ser Leu Ser Pro Gly Lys
 530 535

<210> 13

<211> 1617

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: fusion polypeptide

<220>

<221> CDS

<222> (25)..(1596)

<400> 13

gtcgcacccaa.gctggctagc cacc atg gag aca gac aca ctc ctg cta tgg 51
 Met Glu Thr Asp Thr Leu Leu Leu Trp
 1 5

gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act agt tgt ggg aat 99
 Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Thr Ser Cys Gly Asn
 10 15 20 25

ggt gtg gtt gaa aga gaa gag cag tgt gac tgt gga tcc gta cag cag 147
 Gly Val Val Glu Arg Glu Glu Gln Cys Asp Cys Gly Ser Val Gln Gln
 30 35 40

tgt gaa caa gac gcc tgt tgt ctg ttg aac tgc act cta agg cct ggg 195
 Cys Glu Gln Asp Ala Cys Cys Leu Leu Asn Cys Thr Leu Arg Pro Gly
 45 50 55

gct gcc tgt gct ttt ggg ctt tgt tgc aaa gac tgc aag ttc atg cca 243
 Ala Ala Cys Ala Phe Gly Leu Cys Cys Lys Asp Cys Lys Phe Met Pro
 60 65 70

tca ggg gaa ctc tgt aga caa gag gtc aat gaa tgt gac ctt cca gaa 291
 Ser Gly Glu Leu Cys Arg Gln Glu Val Asn Glu Cys Asp Leu Pro Glu
 75 80 85

tggt tgc aat gga aca tct cat cag tgt cca gaa gat aga tat gtg cag 339
 Trp Cys Asn Gly Thr Ser His Gln Cys Pro Glu Asp Arg Tyr Val Gln
 90 95 100 105

gac ggg atc ccc tgt agt gac agt gcc tac tgc tat caa aag agg tgt 387
 Asp Gly Ile Pro Cys Ser Asp Ser Ala Tyr Cys Tyr Gln Lys Arg Cys
 110 115 120

aat aac cat gac cag cat tgc agg gag att ttt ggt aaa gat gca aaa 435

Asn	Asn	His	Asp	Gln	His	Cys	Arg	Glu	Ile	Phe	Gly	Lys	Asp	Ala	Lys	
			125					130					135			
agt	gca	tct	cag	aat	tgc	tat	aaa	gaa	atc	aac	tct	cag	gga	aac	cgt	483
Ser	Ala	Ser	Gln	Asn	Cys	Tyr	Lys	Glu	Ile	Asn	Ser	Gln	Gly	Asn	Arg	
			140				145					150				
ttt	ggt	cac	tgt	ggt	ata	aat	ggc	aca	aca	tac	cta	aaa	tgt	cat	atc	531
Phe	Gly	His	Cys	Gly	Ile	Asn	Gly	Thr	Thr	Tyr	Leu	Lys	Cys	His	Ile	
			155				160					165				
tct	gat	gtc	ttt	tgt	ggg	aga	ggt	caa	tgt	gag	aat	gtg	aga	gac	att	579
Ser	Asp	Val	Phe	Cys	Gly	Arg	Val	Gln	Cys	Glu	Asn	Val	Arg	Asp	Ile	
170					175					180					185	
cct	ctt	ctc	caa	gat	cat	ttt	act	ttg	cag	cac	act	cat	atc	aat	ggt	627
Pro	Leu	Leu	Gln	Asp	His	Phe	Thr	Leu	Gln	His	Thr	His	Ile	Asn	Gly	
				190					195					200		
gtc	acc	tgc	tgg	ggt	att	gac	tat	cat	tta	agg	atg	aac	ata	tct	gac	675
Val	Thr	Cys	Trp	Gly	Ile	Asp	Tyr	His	Leu	Arg	Met	Asn	Ile	Ser	Asp	
			205					210					215			
att	ggt	gaa	gtg	aaa	gat	ggt	act	gtg	tgt	ggc	cca	gga	aag	atc	tgc	723
Ile	Gly	Glu	Val	Lys	Asp	Gly	Thr	Val	Cys	Gly	Pro	Gly	Lys	Ile	Cys	
		220					225					230				
atc	cat	aag	aag	tgt	gtc	agt	ctg	tct	gtc	ttg	tca	cat	gtc	tgc	ctt	771
Ile	His	Lys	Lys	Cys	Val	Ser	Leu	Ser	Val	Leu	Ser	His	Val	Cys	Leu	
		235					240				245					
cct	gag	acc	tgc	aat	atg	aag	ggg	atc	tgc	aat	aac	aaa	cat	cac	tgc	819
Pro	Glu	Thr	Cys	Asn	Met	Lys	Gly	Ile	Cys	Asn	Asn	Lys	His	His	Cys	
250					255					260					265	
cac	tgt	ggc	tat	ggg	tgg	tcc	cca	ccc	tac	tgc	cag	cac	aga	ggc	tat	867
His	Cys	Gly	Tyr	Gly	Trp	Ser	Pro	Pro	Tyr	Cys	Gln	His	Arg	Gly	Tyr	
				270					275					280		
ggg	ggc	agt	att	gac	agt	ggc	cca	gca	tct	gca	aag	aga	tct	tgt	gac	915
Gly	Gly	Ser	Ile	Asp	Ser	Gly	Pro	Ala	Ser	Ala	Lys	Arg	Ser	Cys	Asp	
			285					290					295			
aaa	act	cac	aca	tgc	cca	ccg	tgc	cca	gca	cct	gaa	gcc	gag	ggc	gcg	963
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Glu	Gly	Ala	
			300				305					310				
ccg	tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	gac	acc	ctc	atg	atc	1011
Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	
			315				320					325				
tcc	cgg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gaa	1059
Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Val	Asp	Val	Ser	His	
330					335					340					Glu	
gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cat	1107
Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	
				350					355					360		
aat	gcc	aag	aca	aag	ccg	cgg	gag	gag	cag	tac	aac	agc	acg	tac	cgg	1155
Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	
			365					370					375			
gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	cag	gac	tgg	ctg	aat	ggc	aag	1203
Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	
			380				385					390				

gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag 1251
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
 395 400 405

 aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac 1299
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
 410 415 420 425

 acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg 1347
 Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
 430 435 440

 acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg 1395
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
 445 450 455

 gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg 1443
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
 460 465 470

 ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac 1491
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
 475 480 485

 aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat 1539
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
 490 495 500 505

 gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg 1587
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
 510 515 520

 ggt aaa tga actagagcgg ccgctacaga t 1617
 Gly Lys

<210> 14

<211> 523

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: fusion polypeptide

<400> 14

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
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 Gly Ser Thr Gly Thr Ser Cys Gly Asn Gly Val Val Glu Arg Glu Glu
 20 25 30
 Gln Cys Asp Cys Gly Ser Val Gln Gln Cys Glu Gln Asp Ala Cys Cys
 35 40 45
 Leu Leu Asn Cys Thr Leu Arg Pro Gly Ala Ala Cys Ala Phe Gly Leu
 50 55 60
 Cys Cys Lys Asp Cys Lys Phe Met Pro Ser Gly Glu Leu Cys Arg Gln
 65 70 75 80
 Glu Val Asn Glu Cys Asp Leu Pro Glu Trp Cys Asn Gly Thr Ser His
 85 90 95
 Gln Cys Pro Glu Asp Arg Tyr Val Gln Asp Gly Ile Pro Cys Ser Asp
 100 105 110
 Ser Ala Tyr Cys Tyr Gln Lys Arg Cys Asn Asn His Asp Gln His Cys
 115 120 125
 Arg Glu Ile Phe Gly Lys Asp Ala Lys Ser Ala Ser Gln Asn Cys Tyr
 130 135 140
 Lys Glu Ile Asn Ser Gln Gly Asn Arg Phe Gly His Cys Gly Ile Asn
 145 150 155 160
 Gly Thr Thr Tyr Leu Lys Cys His Ile Ser Asp Val Phe Cys Gly Arg
 165 170 175

Val Gln Cys Glu Asn Val Arg Asp Ile Pro Leu Leu Gln Asp His Phe
 180 185 190
 Thr Leu Gln His Thr His Ile Asn Gly Val Thr Cys Trp Gly Ile Asp
 195 200 205
 Tyr His Leu Arg Met Asn Ile Ser Asp Ile Gly Glu Val Lys Asp Gly
 210 215 220
 Thr Val Cys Gly Pro Gly Lys Ile Cys Ile His Lys Lys Cys Val Ser
 225 230 235 240
 Leu Ser Val Leu Ser His Val Cys Leu Pro Glu Thr Cys Asn Met Lys
 245 250 255
 Gly Ile Cys Asn Asn Lys His His Cys His Cys Gly Tyr Gly Trp Ser
 260 265 270
 Pro Pro Tyr Cys Gln His Arg Gly Tyr Gly Gly Ser Ile Asp Ser Gly
 275 280 285
 Pro Ala Ser Ala Lys Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro
 290 295 300
 Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro
 305 310 315 320
 Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
 325 330 335
 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
 340 345 350
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 355 360 365
 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 370 375 380
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 385 390 395 400
 Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
 405 410 415
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
 420 425 430
 Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 435 440 445
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 450 455 460
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 465 470 475 480
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 485 490 495
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 500 505 510
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 515 520

<210> 15

<211> 1674

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: fusion polypeptide

<220>

<221> CDS

<222> (25)..(1653)

<400> 15

gtcgacccaa gctggctagc cacc atg gag aca gac aca ctc ctg cta tgg 51
 Met Glu Thr Asp Thr Leu Leu Leu Trp
 1 5

gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act agt tgt ggc aat 99

Val	Leu	Leu	Leu	Trp	Val	Pro	Gly	Ser	Thr	Gly	Thr	Ser	Cys	Gly	Asn	
10					15					20					25	
ggc	ttc	att	gaa	act	gga	gag	gag	tgt	gat	tgt	gga	acc	ccg	gcc	gaa	147
Gly	Phe	Ile	Glu	Thr	Gly	Glu	Glu	Cys	Asp	Cys	Gly	Thr	Pro	Ala	Glu	
				30					35					40		
tgt	gtc	ctt	gaa	gga	gca	gag	tgt	tgt	aag	aaa	tgc	acc	ttg	act	caa	195
Cys	Val	Leu	Glu	Gly	Ala	Glu	Cys	Cys	Lys	Lys	Cys	Thr	Leu	Thr	Gln	
			45					50					55			
gac	tct	caa	tgc	agt	gac	ggt	ctt	tgc	tgt	aaa	aag	tgc	aag	ttt	cag	243
Asp	Ser	Gln	Cys	Ser	Asp	Gly	Leu	Cys	Cys	Lys	Lys	Cys	Lys	Phe	Gln	
		60					65					70				
cct	atg	ggc	act	gtg	tgc	cga	gaa	gca	gta	aat	gat	tgt	gat	att	cgt	291
Pro	Met	Gly	Thr	Val	Cys	Arg	Glu	Ala	Val	Asn	Asp	Cys	Asp	Ile	Arg	
	75					80					85					
gaa	acg	tgc	tca	gga	aat	tca	agc	cag	tgt	gcc	cct	aat	att	cat	aaa	339
Glu	Thr	Cys	Ser	Gly	Asn	Ser	Ser	Gln	Cys	Ala	Pro	Asn	Ile	His	Lys	
	90				95					100					105	
atg	gat	gga	tat	tca	tgt	gat	ggt	gtt	cag	gga	att	tgc	ttt	gga	gga	387
Met	Asp	Gly	Tyr	Ser	Cys	Asp	Gly	Val	Gln	Gly	Ile	Cys	Phe	Gly	Gly	
				110					115					120		
aga	tgc	aaa	acc	aga	gat	aga	caa	tgc	aaa	tac	att	tgg	ggg	caa	aag	435
Arg	Cys	Lys	Thr	Arg	Asp	Arg	Gln	Cys	Lys	Tyr	Ile	Trp	Gly	Gln	Lys	
			125					130					135			
gtg	aca	gca	tca	gac	aaa	tat	tgc	tat	gag	aaa	ctg	aat	att	gaa	ggg	483
Val	Thr	Ala	Ser	Asp	Lys	Tyr	Cys	Tyr	Glu	Lys	Leu	Asn	Ile	Glu	Gly	
		140					145					150				
acg	gag	aag	ggt	aac	tgt	ggg	aaa	gac	aaa	gac	aca	tgg	ata	cag	tgc	531
Thr	Glu	Lys	Gly	Asn	Cys	Gly	Lys	Asp	Lys	Asp	Thr	Trp	Ile	Gln	Cys	
	155					160					165					
aac	aaa	cgg	gat	gtg	ctt	tgt	ggt	tac	ctt	ttg	tgt	acc	aat	att	ggc	579
Asn	Lys	Arg	Asp	Val	Leu	Cys	Gly	Tyr	Leu	Leu	Cys	Thr	Asn	Ile	Gly	
	170				175					180					185	
aat	atc	cca	agg	ctt	gga	gaa	ctc	gat	ggt	gaa	atc	aca	tct	act	tta	627
Asn	Ile	Pro	Arg	Leu	Gly	Glu	Leu	Asp	Gly	Glu	Ile	Thr	Ser	Thr	Leu	
				190					195					200		
gtt	gtg	cag	caa	gga	aga	aca	tta	aac	tgc	agt	ggt	ggg	cat	gtt	aag	675
Val	Val	Gln	Gln	Gly	Arg	Thr	Leu	Asn	Cys	Ser	Gly	Gly	His	Val	Lys	
			205					210					215			
ctt	gaa	gaa	gat	gta	gat	ctt	ggc	tat	gtg	gaa	gat	ggg	aca	cct	tgt	723
Leu	Glu	Glu	Asp	Val	Asp	Leu	Gly	Tyr	Val	Glu	Asp	Gly	Thr	Pro	Cys	
		220					225					230				
ggt	ccc	caa	atg	atg	tgc	tta	gaa	cac	agg	tgt	ctt	cct	gtg	gct	tct	771
Gly	Pro	Gln	Met	Met	Cys	Leu	Glu	His	Arg	Cys	Leu	Pro	Val	Ala	Ser	
	235					240					245					
ttc	aac	ttt	agt	act	tgc	ttg	agc	agt	aaa	gaa	ggc	act	att	tgc	tca	819
Phe	Asn	Phe	Ser	Thr	Cys	Leu	Ser	Ser	Lys	Glu	Gly	Thr	Ile	Cys	Ser	
	250				255					260					265	
gga	aat	gga	gtt	tgc	agt	aat	gag	ctg	aag	tgt	gtg	tgt	aac	aga	cac	867
Gly	Asn	Gly	Val	Cys	Ser	Asn	Glu	Leu	Lys	Cys	Val	Cys	Asn	Arg	His	
				270					275					280		

tgg ata ggt tct gat tgc aac act tac ttc cct cac aat gat gat gca	915
Trp Ile Gly Ser Asp Cys Asn Thr Tyr Phe Pro His Asn Asp Asp Ala	
285 290 295	
aag act ggt atc act ctg tct ggc aat ggt gtt gct ggc acc aat gga	963
Lys Thr Gly Ile Thr Leu Ser Gly Asn Gly Val Ala Gly Thr Asn Gly	
300 305 310	
tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa gcc	1011
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala	
315 320 325	
gag ggc gcg ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc	1059
Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr	
330 335 340 345	
ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg	1107
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val	
350 355 360	
agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg	1155
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val	
365 370 375	
gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc	1203
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser	
380 385 390	
acg tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg	1251
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu	
395 400 405	
aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc	1299
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala	
410 415 420 425	
ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca	1347
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro	
430 435 440	
cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag	1395
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln	
445 450 455	
gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc	1443
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala	
460 465 470	
gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg	1491
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr	
475 480 485	
cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc	1539
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu	
490 495 500 505	
acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc	1587
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser	
510 515 520	
gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc	1635
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser	
525 530 535	
ctg tct ccg ggt aaa tga actagagcgg ccgctacaga t	1674

Leu Ser Pro Gly Lys
540

<210> 16

<211> 542

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: fusion
polypeptide

<400> 16

Met	Glu	Thr	Asp	Thr	Leu	Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro
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Gly	Ser	Thr	Gly	Thr	Ser	Cys	Gly	Asn	Gly	Phe	Ile	Glu	Thr	Gly	Glu
			20					25					30		
Glu	Cys	Asp	Cys	Gly	Thr	Pro	Ala	Glu	Cys	Val	Leu	Glu	Gly	Ala	Glu
		35					40					45			
Cys	Cys	Lys	Lys	Cys	Thr	Leu	Thr	Gln	Asp	Ser	Gln	Cys	Ser	Asp	Gly
	50					55					60				
Leu	Cys	Cys	Lys	Lys	Cys	Lys	Phe	Gln	Pro	Met	Gly	Thr	Val	Cys	Arg
	65				70				75					80	
Glu	Ala	Val	Asn	Asp	Cys	Asp	Ile	Arg	Glu	Thr	Cys	Ser	Gly	Asn	Ser
			85						90					95	
Ser	Gln	Cys	Ala	Pro	Asn	Ile	His	Lys	Met	Asp	Gly	Tyr	Ser	Cys	Asp
			100					105					110		
Gly	Val	Gln	Gly	Ile	Cys	Phe	Gly	Gly	Arg	Cys	Lys	Thr	Arg	Asp	Arg
	115						120						125		
Gln	Cys	Lys	Tyr	Ile	Trp	Gly	Gln	Lys	Val	Thr	Ala	Ser	Asp	Lys	Tyr
	130					135					140				
Cys	Tyr	Glu	Lys	Leu	Asn	Ile	Glu	Gly	Thr	Glu	Lys	Gly	Asn	Cys	Gly
	145				150					155				160	
Lys	Asp	Lys	Asp	Thr	Trp	Ile	Gln	Cys	Asn	Lys	Arg	Asp	Val	Leu	Cys
			165						170					175	
Gly	Tyr	Leu	Leu	Cys	Thr	Asn	Ile	Gly	Asn	Ile	Pro	Arg	Leu	Gly	Glu
		180						185					190		
Leu	Asp	Gly	Glu	Ile	Thr	Ser	Thr	Leu	Val	Val	Gln	Gln	Gly	Arg	Thr
	195						200					205			
Leu	Asn	Cys	Ser	Gly	Gly	His	Val	Lys	Leu	Glu	Glu	Asp	Val	Asp	Leu
	210					215					220				
Gly	Tyr	Val	Glu	Asp	Gly	Thr	Pro	Cys	Gly	Pro	Gln	Met	Met	Cys	Leu
	225				230					235				240	
Glu	His	Arg	Cys	Leu	Pro	Val	Ala	Ser	Phe	Asn	Phe	Ser	Thr	Cys	Leu
			245						250					255	
Ser	Ser	Lys	Glu	Gly	Thr	Ile	Cys	Ser	Gly	Asn	Gly	Val	Cys	Ser	Asn
		260						265					270		
Glu	Leu	Lys	Cys	Val	Cys	Asn	Arg	His	Trp	Ile	Gly	Ser	Asp	Cys	Asn
	275					280						285			
Thr	Tyr	Phe	Pro	His	Asn	Asp	Asp	Ala	Lys	Thr	Gly	Ile	Thr	Leu	Ser
	290					295					300				
Gly	Asn	Gly	Val	Ala	Gly	Thr	Asn	Gly	Ser	Cys	Asp	Lys	Thr	His	Thr
	305				310					315				320	
Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Glu	Gly	Ala	Pro	Ser	Val	Phe
			325						330					335	
Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro
		340						345					350		
Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val
	355						360					365			
Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr
	370					375					380				
Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val
	385				390					395				400	
Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys
			405						410					415	
Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser
			420					425					430		

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Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
      435      440      445
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
      450      455      460
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
465      470      475      480
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
      485      490      495
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
      500      505      510
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
      515      520      525
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
      530      535      540

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<210> 17
 <211> 1668
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: fusion
 polypeptide

<220>
 <221> CDS
 <222> (25)..(1647)

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<400> 17
gtcgacccaa gctggctagc cacc atg gag aca gac aca ctc ctg cta tgg      51
      Met Glu Thr Asp Thr Leu Leu Leu Trp
      1              5

gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act agt tgt gga aat      99
Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Thr Ser Cys Gly Asn
  10              15              20              25

gga tac gtc gaa gct ggg gag gag tgt gat tgt ggt ttt cat gtg gaa      147
Gly Tyr Val Glu Ala Gly Glu Glu Cys Asp Cys Gly Phe His Val Glu
      30              35              40

tgc tat gga tta tgc tgt aag aaa tgt tcc ctc tcc aac ggg gct cac      195
Cys Tyr Gly Leu Cys Cys Lys Lys Cys Ser Leu Ser Asn Gly Ala His
      45              50              55

tgc agc gac ggg ccc tgc tgt aac aat acc tca tgt ctt ttt cag cca      243
Cys Ser Asp Gly Pro Cys Cys Asn Asn Thr Ser Cys Leu Phe Gln Pro
      60              65              70

cga ggg tat gaa tgc cgg gat gct gtg aac gag tgt gat att act gaa      291
Arg Gly Tyr Glu Cys Arg Asp Ala Val Asn Glu Cys Asp Ile Thr Glu
      75              80              85

tat tgt act gga gac tct ggt cag tgc cca cca aat ctt cat aag caa      339
Tyr Cys Thr Gly Asp Ser Gly Gln Cys Pro Pro Asn Leu His Lys Gln
      90              95              100              105

gac gga tat gca tgc aat caa aat cag ggc cgc tgc tac aat ggc gag      387
Asp Gly Tyr Ala Cys Asn Gln Asn Gln Gly Arg Cys Tyr Asn Gly Glu
      110              115              120

tgc aag gcc aga gac aac cag tgt cag tac atc tgg gga aca aag gct      435
Cys Lys Ala Arg Asp Asn Gln Cys Gln Tyr Ile Trp Gly Thr Lys Ala
      125              130              135

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gca ggg tct gac aag ttc tgc tat gaa aag ctg aat aca gaa ggc act	483
Ala Gly Ser Asp Lys Phe Cys Tyr Glu Lys Leu Asn Thr Glu Gly Thr	
140 145 150	
gag aag gga aac tgc ggg aag gat gga gac cgg tgg att cag tgc agc	531
Glu Lys Gly Asn Cys Gly Lys Asp Gly Asp Arg Trp Ile Gln Cys Ser	
155 160 165	
aaa cat gat gtg ttc tgt gga ttc tta ctc tgt acc aat ctt act cga	579
Lys His Asp Val Phe Cys Gly Phe Leu Leu Cys Thr Asn Leu Thr Arg	
170 175 180 185	
gct cca cgt att ggt caa ctt cag ggt gag atc att cca act tcc ttc	627
Ala Pro Arg Ile Gly Gln Leu Gln Gly Glu Ile Ile Pro Thr Ser Phe	
190 195 200	
tac cat caa ggc cgg gtg att gac tgc agt ggt gcc cat gta gtt tta	675
Tyr His Gln Gly Arg Val Ile Asp Cys Ser Gly Ala His Val Val Leu	
205 210 215	
gat gat gat acg gat gtg ggc tat gta gaa gat gga acg cca tgt ggc	723
Asp Asp Asp Thr Asp Val Gly Tyr Val Glu Asp Gly Thr Pro Cys Gly	
220 225 230	
ccg tct atg atg tgt tta gat cgg aag tgc cta caa att caa gcc cta	771
Pro Ser Met Met Cys Leu Asp Arg Lys Cys Leu Gln Ile Gln Ala Leu	
235 240 245	
aat atg agc agc tgt cca ctc gat tcc aag ggt aaa gtc tgt tcg ggc	819
Asn Met Ser Ser Cys Pro Leu Asp Ser Lys Gly Lys Val Cys Ser Gly	
250 255 260 265	
cat ggg gtg tgt agt aat gaa gcc acc tgc att tgt gat ttc acc tgg	867
His Gly Val Cys Ser Asn Glu Ala Thr Cys Ile Cys Asp Phe Thr Trp	
270 275 280	
gca ggg aca gat tgc agt atc cgg gat cca gtt agg aac ctt cac ccc	915
Ala Gly Thr Asp Cys Ser Ile Arg Asp Pro Val Arg Asn Leu His Pro	
285 290 295	
ccc aag gat gaa gga ccc aag ggt cct agt gcc acc aat aga tct tgt	963
Pro Lys Asp Glu Gly Pro Lys Gly Pro Ser Ala Thr Asn Arg Ser Cys	
300 305 310	
gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa gcc gag ggc	1011
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly	
315 320 325	
gcg ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg	1059
Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met	
330 335 340 345	
atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac	1107
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His	
350 355 360	
gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg	1155
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val	
365 370 375	
cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac	1203
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr	
380 385 390	
cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc	1251

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Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
 395                                400                                405

aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc 1299
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
410                                415                                420                                425

gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg 1347
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
                                430                                435                                440

tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc 1395
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
                                445                                450                                455

ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag 1443
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
                                460                                465                                470

tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc 1491
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                                475                                480                                485

gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg 1539
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
490                                495                                500                                505

gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg 1587
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                                510                                515                                520

cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct 1635
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
                                525                                530                                535

ccg ggt aaa tga actagagcgg ccgctacaga t 1668
Pro Gly Lys
                                540

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<210> 18

<211> 540

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: fusion polypeptide

<400> 18

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Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1                                5                                10                                15
Gly Ser Thr Gly Thr Ser Cys Gly Asn Gly Tyr Val Glu Ala Gly Glu
                                20                                25                                30
Glu Cys Asp Cys Gly Phe His Val Glu Cys Tyr Gly Leu Cys Cys Lys
                                35                                40                                45
Lys Cys Ser Leu Ser Asn Gly Ala His Cys Ser Asp Gly Pro Cys Cys
                                50                                55                                60
Asn Asn Thr Ser Cys Leu Phe Gln Pro Arg Gly Tyr Glu Cys Arg Asp
65                                70                                75                                80
Ala Val Asn Glu Cys Asp Ile Thr Glu Tyr Cys Thr Gly Asp Ser Gly
                                85                                90                                95
Gln Cys Pro Pro Asn Leu His Lys Gln Asp Gly Tyr Ala Cys Asn Gln
                                100                                105                                110
Asn Gln Gly Arg Cys Tyr Asn Gly Glu Cys Lys Ala Arg Asp Asn Gln
115                                120                                125
Cys Gln Tyr Ile Trp Gly Thr Lys Ala Ala Gly Ser Asp Lys Phe Cys
130                                135                                140

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Tyr Glu Lys Leu Asn Thr Glu Gly Thr Glu Lys Gly Asn Cys Gly Lys
145          150          155          160
Asp Gly Asp Arg Trp Ile Gln Cys Ser Lys His Asp Val Phe Cys Gly
          165          170          175
Phe Leu Leu Cys Thr Asn Leu Thr Arg Ala Pro Arg Ile Gly Gln Leu
          180          185          190
Gln Gly Glu Ile Ile Pro Thr Ser Phe Tyr His Gln Gly Arg Val Ile
          195          200          205
Asp Cys Ser Gly Ala His Val Val Leu Asp Asp Asp Thr Asp Val Gly
          210          215          220
Tyr Val Glu Asp Gly Thr Pro Cys Gly Pro Ser Met Met Cys Leu Asp
225          230          235          240
Arg Lys Cys Leu Gln Ile Gln Ala Leu Asn Met Ser Ser Cys Pro Leu
          245          250          255
Asp Ser Lys Gly Lys Val Cys Ser Gly His Gly Val Cys Ser Asn Glu
          260          265          270
Ala Thr Cys Ile Cys Asp Phe Thr Trp Ala Gly Thr Asp Cys Ser Ile
          275          280          285
Arg Asp Pro Val Arg Asn Leu His Pro Pro Lys Asp Glu Gly Pro Lys
          290          295          300
Gly Pro Ser Ala Thr Asn Arg Ser Cys Asp Lys Thr His Thr Cys Pro
305          310          315          320
Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe
          325          330          335
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
          340          345          350
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
          355          360          365
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
          370          375          380
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
385          390          395          400
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
          405          410          415
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
          420          425          430
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
          435          440          445
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
          450          455          460
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
465          470          475          480
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
          485          490          495
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
          500          505          510
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
          515          520          525
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
          530          535          540

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<210> 19

<211> 3

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Consensus
binding motif

<400> 19

Arg Gly Asp

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<210> 20
 <211> 67
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: consensus
 disintegrin domain

<220>
 <221> VARIANT
 <222> (5)..(9)
 <223> 3-5 varying residues in a consensus sequence

<220>
 <221> VARIANT
 <222> (11)..(16)
 <223> 3-6 varying residues in a consensus sequence

<220>
 <221> VARIANT
 <222> (19)..(22)
 <223> 2-4 varying residues in a consensus sequence

<220>
 <221> VARIANT
 <222> (24)..(30)
 <223> 7 varying residues in a consensus sequence

<220>
 <221> VARIANT
 <222> (32)..(37)
 <223> 4-6 varying residues in a consensus sequence

<220>
 <221> VARIANT
 <222> (40)..(43)
 <223> 2-4 varying residues in a consensus sequence

<220>
 <221> VARIANT
 <222> (45)..(52)
 <223> 8 varying residues in a consensus sequence

<220>
 <221> VARIANT
 <222> (54)..(60)
 <223> 5-7 varying residues in a consensus sequence

<220>
 <221> VARIANT
 <222> (62)..(66)
 <223> 3-5 varying residues in a consensus sequence

<400> 20
 Cys Asp Cys Gly Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa
 1 5 10 15
 Cys Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa
 20 25 30
 Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
 35 40 45
 Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
 50 55 60

Xaa Xaa Cys
65

<210> 21
<211> 1725
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: fusion
polypeptide

<220>
<221> CDS
<222> (118)..(1704)

<400> 21
gggttttccc agtcacgacg ttgtaaaacg acggccagtg aattgtaata cgactcacta 60
tagggcgaat tgggtaccgg gccccccctc gaggtcgacc caagctggct agccacc 117
atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca 165
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15
ggt tcc act ggt act agt tgt ggg aat ggt gtg gtt gaa gaa gga gaa 213
Gly Ser Thr Gly Thr Ser Cys Gly Asn Gly Val Val Glu Glu Gly Glu
20 25 30
gag tgt gac tgt gga cct tta aag cat tgt gca aaa gat ccc tgc tgt 261
Glu Cys Asp Cys Gly Pro Leu Lys His Cys Ala Lys Asp Pro Cys Cys
35 40 45
ctg tca aat tgc act ctg act gat ggt tct act tgt gct ttt ggg ctt 309
Leu Ser Asn Cys Thr Leu Thr Asp Gly Ser Thr Cys Ala Phe Gly Leu
50 55 60
tgt tgc aaa gac tgc aag ttc cta cca tca ggg aaa gtg tgt aga aag 357
Cys Cys Lys Asp Cys Lys Phe Leu Pro Ser Gly Lys Val Cys Arg Lys
65 70 75 80
gag gtc aat gaa tgt gat ctt cca gag tgg tgc aat ggt act tcc cat 405
Glu Val Asn Glu Cys Asp Leu Pro Glu Trp Cys Asn Gly Thr Ser His
85 90 95
aag tgc cca gat gac ttt tat gtg gaa gat gga att ccc tgt aag gag 453
Lys Cys Pro Asp Asp Phe Tyr Val Glu Asp Gly Ile Pro Cys Lys Glu
100 105 110
agg ggc tac tgc tat gaa aag agc tgt cat gac cgc aat gaa cag tgt 501
Arg Gly Tyr Cys Tyr Glu Lys Ser Cys His Asp Arg Asn Glu Gln Cys
115 120 125
agg agg att ttt ggt gca ggc gca aat act gca agt gag act tgc tac 549
Arg Arg Ile Phe Gly Ala Gly Ala Asn Thr Ala Ser Glu Thr Cys Tyr
130 135 140
aaa gaa ttg aac acc tta ggt gac cgt gtt ggt cac tgt ggt atc aaa 597
Lys Glu Leu Asn Thr Leu Gly Asp Arg Val Gly His Cys Gly Ile Lys
145 150 155 160
aat gct aca tat ata aag tgt aat atc tca gat gtc cag tgt gga aga 645
Asn Ala Thr Tyr Ile Lys Cys Asn Ile Ser Asp Val Gln Cys Gly Arg
165 170 175

att cag tgt gag aat gtg aca gaa att ccc aat atg agt gat cat act	693
Ile Gln Cys Glu Asn Val Thr Glu Ile Pro Asn Met Ser Asp His Thr	
180 185 190	
act gtg cat tgg gct cgc ttc aat gac ata atg tgc tgg agt act gat	741
Thr Val His Trp Ala Arg Phe Asn Asp Ile Met Cys Trp Ser Thr Asp	
195 200 205	
tac cat ttg ggg atg aag gga cct gat att ggt gaa gtg aaa gat gga	789
Tyr His Leu Gly Met Lys Gly Pro Asp Ile Gly Glu Val Lys Asp Gly	
210 215 220	
aca gag tgt ggg ata gat cat ata tgc atc cac agg cac tgt gtc cat	837
Thr Glu Cys Gly Ile Asp His Ile Cys Ile His Arg His Cys Val His	
225 230 235 240	
ata acc atc ttg aat agt aat tgc tca cct gca ttt tgt aac aag agg	885
Ile Thr Ile Leu Asn Ser Asn Cys Ser Pro Ala Phe Cys Asn Lys Arg	
245 250 255	
ggc atc tgc aac aat aaa cat cac tgc cat tgc aat tat ctg tgg gac	933
Gly Ile Cys Asn Asn Lys His His Cys His Cys Asn Tyr Leu Trp Asp	
260 265 270	
cct ccc aac tgc ctg ata aaa ggc tat gga ggt agt gtt gac agt ggc	981
Pro Pro Asn Cys Leu Ile Lys Gly Tyr Gly Gly Ser Val Asp Ser Gly	
275 280 285	
cca ccc cct aag aga aag aag aaa aag aag aga tct tgt gac aaa act	1029
Pro Pro Pro Lys Arg Lys Lys Lys Lys Lys Arg Ser Cys Asp Lys Thr	
290 295 300	
cac aca tgc cca ccg tgc cca gca cct gaa gcc gag ggc gcg ccg tca	1077
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser	
305 310 315 320	
gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg	1125
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg	
325 330 335	
acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct	1173
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro	
340 345 350	
gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc	1221
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala	
355 360 365	
aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg gtc	1269
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val	
370 375 380	
agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac	1317
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr	
385 390 395 400	
aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc	1365
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr	
405 410 415	
atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg	1413
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu	
420 425 430	
ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc	1461

Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	
		435					440					445				
ctg	gtc	aaa	ggc	ttc	tat	ccc	agc	gac	atc	gcc	gtg	gag	tgg	gag	agc	1509
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	
	450					455					460					
aat	ggg	cag	ccg	gag	aac	aac	tac	aag	acc	acg	cct	ccc	gtg	ctg	gac	1557
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Pro	Pro	Val	Leu	Asp		
465					470					475				480		
tcc	gac	ggc	tcc	ttc	ttc	ctc	tac	agc	aag	ctc	acc	gtg	gac	aag	agc	1605
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	
				485					490					495		
agg	tgg	cag	cag	ggg	aac	gtc	ttc	tca	tgc	tcc	gtg	atg	cat	gag	gct	1653
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	
		500						505					510			
ctg	cac	aac	cac	tac	acg	cag	aag	agc	ctc	tcc	ctg	tct	ccg	ggg	aaa	1701
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	
		515					520					525				
tga	actagagcgg	ccgctacaga	t													1725

<210> 22

<211> 528

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: fusion polypeptide

<400> 22

Met	Glu	Thr	Asp	Thr	Leu	Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro	
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Gly	Ser	Thr	Gly	Thr	Ser	Cys	Gly	Asn	Gly	Val	Val	Glu	Glu	Gly	Glu	
			20					25					30			
Glu	Cys	Asp	Cys	Gly	Pro	Leu	Lys	His	Cys	Ala	Lys	Asp	Pro	Cys	Cys	
		35					40					45				
Leu	Ser	Asn	Cys	Thr	Leu	Thr	Asp	Gly	Ser	Thr	Cys	Ala	Phe	Gly	Leu	
	50					55					60					
Cys	Cys	Lys	Asp	Cys	Lys	Phe	Leu	Pro	Ser	Gly	Lys	Val	Cys	Arg	Lys	
	65				70					75				80		
Glu	Val	Asn	Glu	Cys	Asp	Leu	Pro	Glu	Trp	Cys	Asn	Gly	Thr	Ser	His	
			85						90					95		
Lys	Cys	Pro	Asp	Asp	Phe	Tyr	Val	Glu	Asp	Gly	Ile	Pro	Cys	Lys	Glu	
		100						105					110			
Arg	Gly	Tyr	Cys	Tyr	Glu	Lys	Ser	Cys	His	Asp	Arg	Asn	Glu	Gln	Cys	
	115						120					125				
Arg	Arg	Ile	Phe	Gly	Ala	Gly	Ala	Asn	Thr	Ala	Ser	Glu	Thr	Cys	Tyr	
	130				135					140						
Lys	Glu	Leu	Asn	Thr	Leu	Gly	Asp	Arg	Val	Gly	His	Cys	Gly	Ile	Lys	
	145				150				155					160		
Asn	Ala	Thr	Tyr	Ile	Lys	Cys	Asn	Ile	Ser	Asp	Val	Gln	Cys	Gly	Arg	
			165					170					175			
Ile	Gln	Cys	Glu	Asn	Val	Thr	Glu	Ile	Pro	Asn	Met	Ser	Asp	His	Thr	
		180						185					190			
Thr	Val	His	Trp	Ala	Arg	Phe	Asn	Asp	Ile	Met	Cys	Trp	Ser	Thr	Asp	
	195						200					205				
Tyr	His	Leu	Gly	Met	Lys	Gly	Pro	Asp	Ile	Gly	Glu	Val	Lys	Asp	Gly	
	210					215					220					
Thr	Glu	Cys	Gly	Ile	Asp	His	Ile	Cys	Ile	His	Arg	His	Cys	Val	His	
	225				230					235				240		
Ile	Thr	Ile	Leu	Asn	Ser	Asn	Cys	Ser	Pro	Ala	Phe	Cys	Asn	Lys	Arg	
			245						250					255		

Gly	Ile	Cys	Asn	Asn	Lys	His	His	Cys	His	Cys	Asn	Tyr	Leu	Trp	Asp		
			260					265					270				
Pro	Pro	Asn	Cys	Leu	Ile	Lys	Gly	Tyr	Gly	Gly	Ser	Val	Asp	Ser	Gly		
		275					280					285					
Pro	Pro	Pro	Lys	Arg	Lys	Lys	Lys	Lys	Arg	Ser	Cys	Asp	Lys	Thr			
		290				295				300							
His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Glu	Gly	Ala	Pro	Ser		
305					310				315						320		
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg		
			325					330						335			
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro		
			340					345					350				
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala		
		355					360					365					
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val		
		370				375					380						
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr		
385					390					395					400		
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr		
			405					410						415			
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu		
			420					425					430				
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys		
		435					440					445					
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser		
		450				455					460						
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp		
465				470					475						480		
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser		
			485					490						495			
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala		
		500						505					510				
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys		
		515					520					525					

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(54) Title: INTEGRIN ANTAGONISTS

(57) Abstract: The present invention provides methods and compositions for inhibiting the biological activity of integrins, for inhibiting endothelial cell migration, and for inhibiting angiogenesis. In particular, the invention provides compositions comprising ADAM disintegrin domains and methods for using said compositions. In preferred embodiments the methods and compositions of the invention are used to inhibit angiogenesis and to treat diseases or conditions mediated by angiogenesis.

INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 01/05701

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/64 C12N15/57 A61K38/16 A61P35/00 A61P37/00
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, WPI Data, PAJ, SCISEARCH, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHLUESENER HERMANN J: "The disintegrin domain of ADAM 8 enhances protection against rat experimental autoimmune encephalomyelitis, neuritis and uveitis by a polyvalent autoantigen vaccine." JOURNAL OF NEUROIMMUNOLOGY, vol. 87, no. 1-2, 1 July 1998 (1998-07-01), pages 197-202, XP000926791 ISSN: 0165-5728 page 199 -page 201; figure 2A --- -/--	1-3, 16, 17, 26

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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- *E* earlier document but published on or after the international filing date
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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Y document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 01/05701

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATH DEEPA ET AL: "Interaction of metargidin (ADAM-15) with alphavbeta3 and alpha5beta1 integrins on different haemopoietic cells." JOURNAL OF CELL SCIENCE. vol. 112, no. 4, February 1999 (1999-02), pages 579-587, XP002186267 LONDON GB ISSN: 0021-9533 cited in the application the whole document, especially page 586, column 1	1-3, 7-18,27, 31,33-41
Y A	---	4 35-42
X	ZHANG XI-PING ET AL: "Specific interaction of the recombinant disintegrin-like domain of MDC-15 (metargidin, ADAM-15) with integrin alphavbeta3." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 13, 27 March 1998 (1998-03-27), pages 7345-7350, XP002186268 WASHINGTON US ISSN: 0021-9258 the whole document, especially page 7349, column 2, paragraph 2	1-3, 9-18,27, 31,33
Y	---	4
	SHEU J-R ET AL: "Inhibition of angiogenesis in vitro and in vivo: comparison of the relative activities of triflavin, an Arg-Gly-Asp-containing peptide and anti-alphavbeta3 integrin monoclonal antibody" BBA - GENERAL SUBJECTS, ELSEVIER SCIENCE PUBLISHERS, NL, vol. 1336, no. 3, 20 October 1997 (1997-10-20), pages 445-454, XP004276037 ISSN: 0304-4165 abstract --- -/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/05701

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TSELEPIS VICKY H ET AL: "An RGD to LDV motif conversion within the disintegrin kistrin generates an integrin antagonist that retains potency but exhibits altered receptor specificity: Evidence for a functional equivalence of acidic integrin-binding motifs" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 272, no. 34, 1997, pages 21341-21348, XP002149905 ISSN: 0021-9258 the whole document	4
A	WO 99 41388 A (IMMUNEX CORP) 19 August 1999 (1999-08-19) cited in the application the whole document	1-42
A	WO 99 23228 A (IMMUNEX CORP) 14 May 1999 (1999-05-14) cited in the application page 6, paragraph 2 page 8, paragraph 2	1-42
A	WO 99 36549 A (IMMUNEX CORP) 22 July 1999 (1999-07-22) cited in the application page 4, line 24 - line 30 page 7, line 25 -page 8, line 26	1-42
P,X	WO 00 43493 A (HUMAN GENOME SCIENCES INC) 27 July 2000 (2000-07-27) page 13, line 3 page 17, line 6 - line 7 page 196, line 31 -page 204, line 33 page 227 -page 234 examples 10,39,41-43,49	1-9, 11-29, 31,32, 34-42
E	WO 01 74857 A (BRISTOL-MYERS SQUIBB CO) 11 October 2001 (2001-10-11) page 4, line 26 -page 6, line 16 page 7, line 11 -page 8, line 26 page 14, line 17 - line 34; example 12	1-18,20, 27,28, 30-42

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3, 18-20, 26 completely and 5-17, 21-25 partly

A method of antagonizing the binding of an integrin to its ligand, in vitro or in vivo, by administering an effective amount of an ADAM disintegrin domain polypeptide

2. Claims: 4, 28, 29 completely and 5-17, 21-25, 27 partly

A method of inhibiting angiogenesis in a mammal comprising administering an ADAM disintegrin domain polypeptide which does not contain a RGD sequence

3. Claim : 27 partly and 30 completely

A method for inhibiting the biological activity of alphaIIbetaI integrin comprising contacting the integrin with an ADAM-23 disintegrin polypeptide

4. Claim : 27 partly and 31 completely

A method for inhibiting the biological activity of alphaVbetaI integrin comprising contacting the integrin with an ADAM disintegrin polypeptide and the ADAM is ADAM-15, -21, -22 or -23

5. Claim : 27 partly and 32 completely

A method for inhibiting the biological activity of alphaVIbetaI or alphaVIbetaIV integrin comprising contacting the integrin with an ADAM disintegrin polypeptide and the ADAM is ADAM-10, -17, -22 or -23

6. Claim : 27 partly and 33 completely

A method for inhibiting the biological activity of alphaVbetaV integrin comprising contacting the integrin with an ADAM disintegrin polypeptide and the ADAM is ADAM-10, -15 or -23

7. Claims: 34-42

Methods for identifying compounds that modulate integrin biological activity

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-10 and 15-26 relate to a method defined by reference to the use of a compound having a desirable characteristic or property, namely having an "ADAM disintegrating domain". The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the subject-matter of claims 11-14, insofar as those claims refer to amino acid or nucleotide sequences as identified in the sequence listing since fragments (claim 11b, 13b), variants (claim 11c) fusion proteins (claim 11d) or hybridizing nucleic acids (claim 14 c) retaining at least one 'ADAMdis' activity are not disclosed as well.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/05701

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9941388	A	19-08-1999	AU 3290899 A EP 1054982 A2 WO 9941388 A2	30-08-1999 29-11-2000 19-08-1999
WO 9923228	A	14-05-1999	AU 1287699 A EP 1027442 A1 JP 2001521742 T WO 9923228 A1	24-05-1999 16-08-2000 13-11-2001 14-05-1999
WO 9936549	A	22-07-1999	AU 2221999 A EP 1045914 A1 WO 9936549 A1	02-08-1999 25-10-2000 22-07-1999
WO 0043493	A	27-07-2000	AU 3212400 A WO 0043493 A2	07-08-2000 27-07-2000
WO 0174857	A	11-10-2001	WO 0174857 A2	11-10-2001